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ABSTRACT

This publication contains a collection of research papers designed to be used as a supplement to the textbook in a high school biology class. The material can be used to enrich the curriculum by stimulating critical thinking and by making high school students aware of the ways in which scientists work. Some of the papers have been condensed or paraphrased so that a high school student could use the publication as part of an independent study program. The papers are grouped under the following headings: Unity among Living Things, Biology of Man, Biology of Plants, The Conquest of Disease, The Origins of Life, The Origins of New Organisms, The Transfer of Traits, and Observation and Experimentation. Also included is a two-page figure spanning the period 1550 - 1950 and showing when famous scientists lived along with contemporaries who achieved fame in other areas of endeavor. (PEB)

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BIOLOGY...

its people and its papers

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BIOLOGY...
its people and its papers

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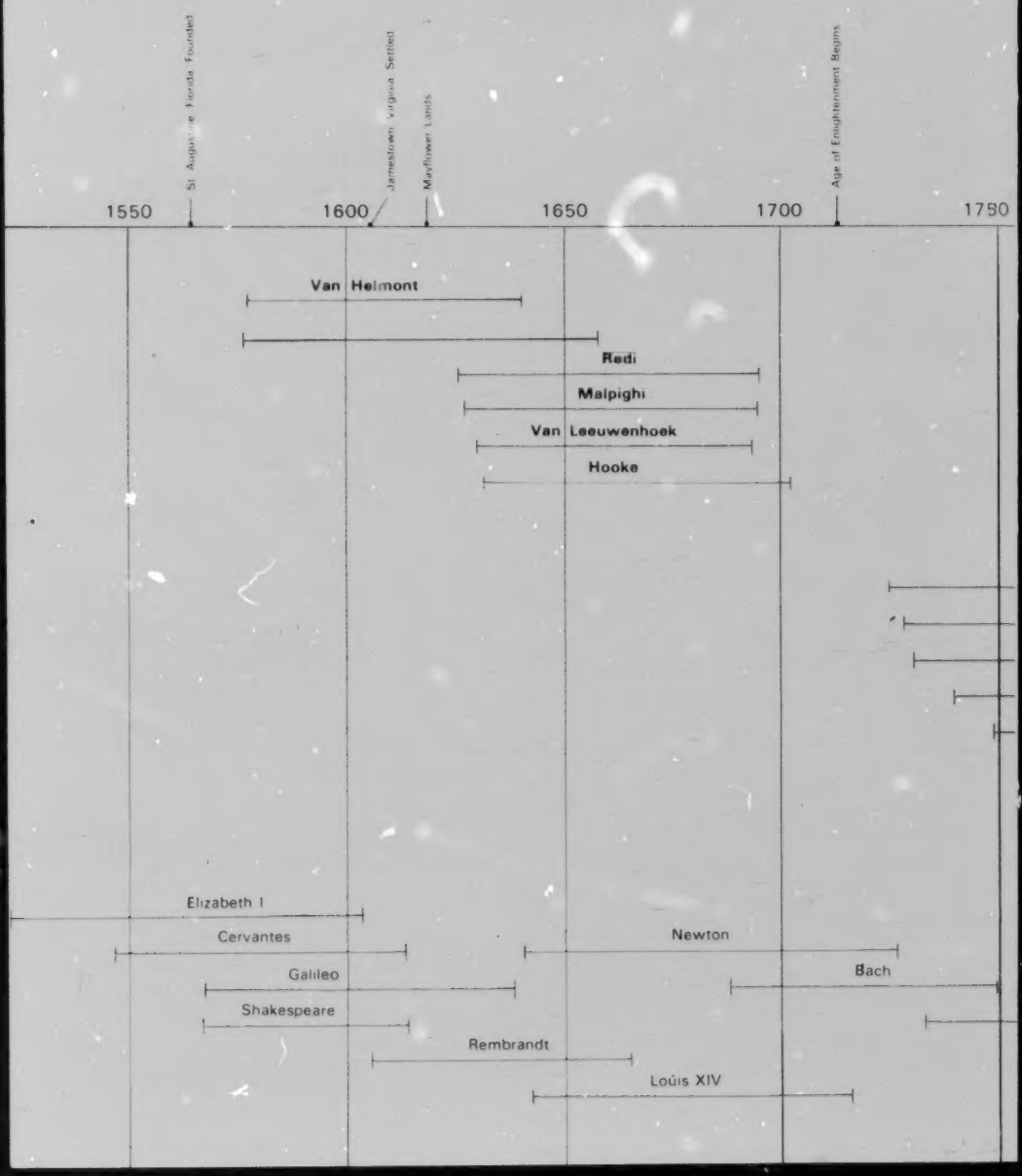

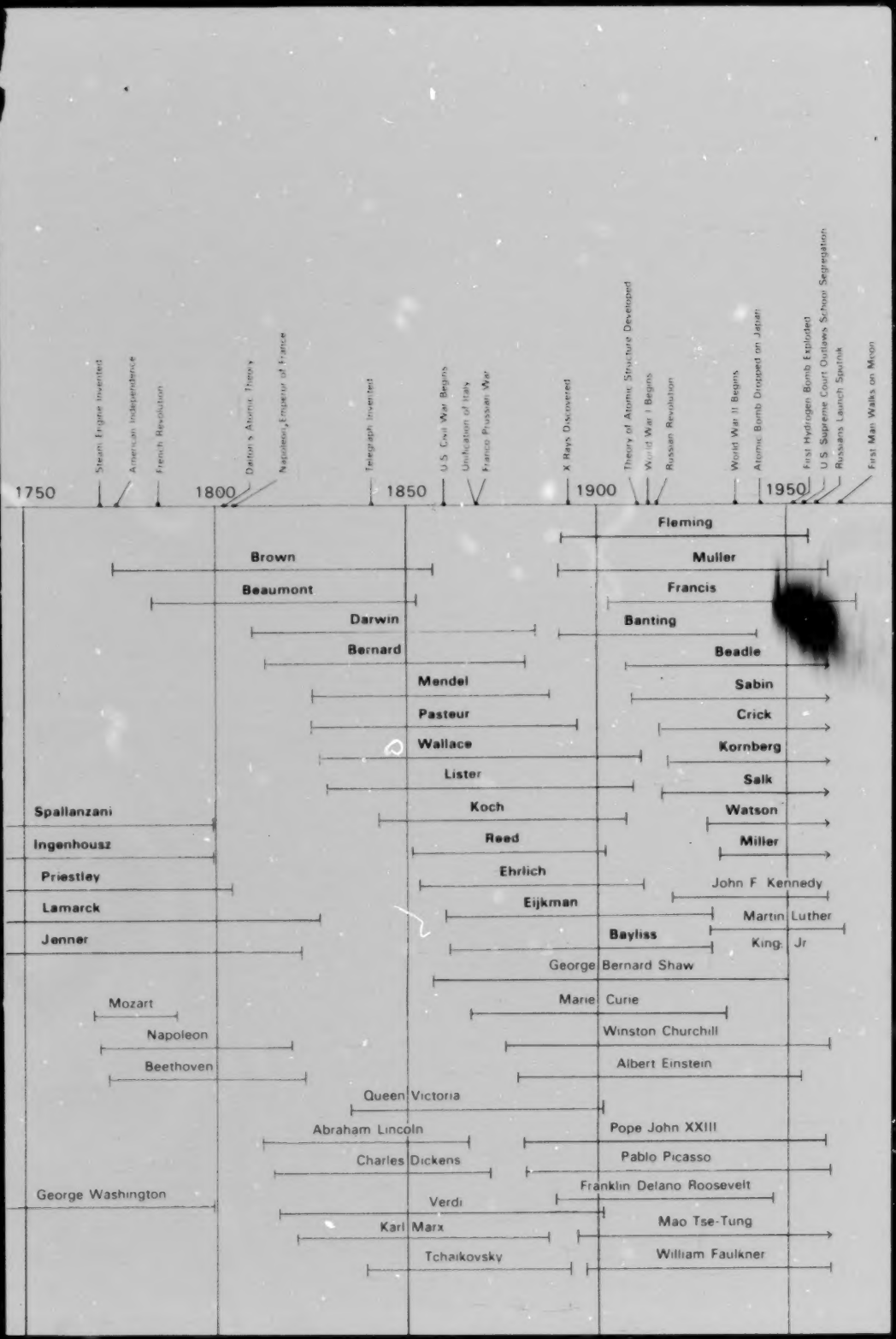
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Napoleon, Emperor of France
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Queen Victoria
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Charles Dickens
Pablo Picasso
Franklin Delano Roosevelt
George Washington
Verdi
Mao Tse-Tung
Karl Marx
Tchaikovsky
William Faulkner

INTRODUCTION

About Science and Scientists . . . .

What are scientists like? This question cannot be answered easily, because people with differing personalities, backgrounds, and abilities have made important contributions to the world of science.

Claude Bernard was born to poor peasant parents, while Charles Darwin was born into a wealthy, scientifically oriented family. Anton van Leeuwenhoek was an amateur observer, William Beaumont a frontier doctor, and Gregor Mendel a monk. Francesco Redi was a poet, William Harvey and Marcello Malpighi served a king and a pope, and Herman Muller and Joseph Priestley were political activists. These ten scientists represent six countries and several different religions.

Some scientists had great experimental ability, some used keen powers of observation, whereas others were great thinkers. And all of them had their own individual way of attacking a scientific problem. So the manner in which science proceeds is quite different from the series of steps often listed in textbooks and called "the scientific method."

In order to appreciate the work of the scientist, it is important to know something about the period in which he lived. Throughout history scientists either challenged widely accepted beliefs or extended knowledge beyond that known at the time.

By learning about the lives of biologists and by reading their original research papers, you will get a picture of what science is and of how scientists work.

To the Teacher:

There is genuine concern on the part of scientists, science educators, and science teachers that students develop insights into the methods of science. The scientific literature contains considerable evidence to indicate that the use of historical materials and contact with original research papers can play a role in inculcating a conception of what science is about, how science works, and what the real character of the scientific enterprise is.

This collection of research papers should be used as a supplement to the regular biology textbook. It can enrich the curriculum by stimulating critical thinking and by making the students aware of the ways in which scientists work.

The papers chosen can serve as a framework around which lessons can be developed, or they can be used as part of an independent study program. For the use of high school students the papers have been somewhat condensed or paraphrased to clarify the language.

Each guide accompanying a paper is designed to highlight aspects of the scientific process. Students are asked to identify problems, hypotheses, and assumptions. They are also challenged to analyze experimental procedures and to draw conclusions based upon the data. Some of the guide questions are relatively simple; others, more difficult. With proper teacher guidance, the average student should be able to complete the guides successfully.

An important aspect of this book is its interdisciplinary nature. Many of the papers correlate with specific historical events. For example, no discussion of the building of the Panama Canal would be complete without considering the work of Walter Reed. Some papers may serve to illustrate the literary style of the times in which they were written.

In examining these papers one observes trends in the nature of the scientific enterprise. The early biologist was an amateur who pursued his investigations as a hobby, whereas the modern biologist is a highly specialized professional. Modern research papers are very technical, impersonal, and objective. In the earlier papers, the investigators were inclined to reveal their thoughts and emotions. Today we see a number of research teams, directing their attention to biochemical and biophysical problems. In the past, biologists worked alone and needed little knowledge of other scientific disciplines.

These ideas, and others that the teacher may develop, can stimulate or emerge from class discussion, and can help make biology more exciting to the student.

UNITY AMONG LIVING THINGS

Robert Hooke—An Excursion into the Microscopic World

In tracing the history of science, one can identify many major "break throughs" in the evolution of scientific ideas and theories. Usually these followed the development of specific instruments which made possible many new discoveries.

Ancient man used glass spheres to make lenses. Arabian mathematicians of the tenth century used them in their calculations, and lens construction began in Europe in the thirteenth century. In time, eyeglasses and magnifying glasses were invented. In the sixteenth century, Leonardo DaVinci also experimented with lenses.

Shortly before 1600, microscopes were constructed in Holland. The Italian astronomer Galileo and the Dutch investigator Leeuwenhoek both fashioned microscopes, which freed naturalists from depending upon their unaided senses.

In addition, these new instruments were able to extend the quest for new knowledge beyond such usual sources as books and lectures. Scientists were now able to use their own eyes and their own interpretations to make independent and unbiased observations.

The idea of being able to see the smallest of things must have appealed to Robert Hooke (1635-1703). Despite poor health as a child, which interfered with his education, this English scientist was able to make significant contributions to the physical sciences, in addition to being one of the great early microscopists. He showed great originality in devising such new instruments as air pumps, diving bells, rain gauges, and adding machines. Hooke also possessed an inquisitive mind which led him to investigate concepts of gravity and mechanics.

Both he and Sir Isaac Newton were members of the Royal Society. Hooke once criticized one of Newton's experiments on

light when it was presented to the Society. These two great men developed a dislike for each other as a result of that incident.

In fact, Hooke was not generally liked by his fellow scientists because of his irritable temperament. However, Hooke was a versatile investigator who improved upon previous microscopes by using two lenses in combination, thereby developing a compound microscope which he used to make a number of significant observations. In this paper Hooke describes the appearance of a thin piece of cork as viewed through his microscope.

THE MICROSCOPIC STRUCTURE OF CORK

ROBERT HOOKE (1665)

I took a good clear piece of cork, and with a pen-knife sharpened as keen as a razor, I cut a piece of it off, and thereby left a surface of it exceeding smooth; then examining it very diligently with a microscope, me thought I could perceive it to appear a little porous, but I could not so plainly distinguish as to be sure that they were pores, much less what Figure they were of. But judging from the lightness and yielding quality of Cork, that certainly the texture could not be so curious but that possible, if I could use some further diligence, I might find it to be discernible with a Microscope, I with the same sharp pen-knife, cut off from the former smooth surface an exceeding thin piece of it, and placing it on a black object plate, because it was itself a white body, and casting light upon it with a plane-convex glass I could exceedingly plainly perceive it to be all perforated and porous, much like a Honey-comb, but that the pores of it were not regular.

I no sooner discerned these (which were indeed the first microscopical pores I ever saw, and perhaps, that were ever seen, for I had not met with any Writer or Person, that had made any mention of them before this), but me thought I had with the discovery of them, presently hinted to me the true and intelligible reason of all the Phenomena of Cork.

But to return to our Observation, I told several lines of these pores and found that there were usually about three-score of these small cells placed end ways in the eighteenth part of an inch, whence I concluded that there must be near eleven hundred of them or somewhat more than a thousand in the length of an Inch, and therefore in a square inch above a Million or 1166400 and in a Cubic Inch, about twelve hundred Million or 1259712000 a thing almost incredible did not our Microscope assure us of it by ocular demonstration.

Nor is this kind of Texture peculiar to Cork only; for upon examination into my Microscope, I have found that the pith of an Elder, or almost any other Tree, have much the same kind of Schematism, as I have lately shown that of Cork, save only that here the pores ranged the long-ways, whereas in the Cork they are Transverse.

Micrographia London. 1665. And in Philosophical Transactions of the Royal Society. London. 1681-1682.

Guide to Paper by Robert Hooke

1. *Why did Hooke use "an exceedingly thin piece" of cork?*
2. *What term would one use today to describe the "microscopical pores" observed by Hooke?*
3. *Why did Hooke feel that he was the first person to observe these microscopical pores?*
4. *What were Hooke's conclusions regarding the size of each individual cell?*

Anton van Leeuwenhoek—An Amateur's Wanderings Through the Natural World

In the early days of scientific development, personal curiosity and devotion to investigation enabled many amateurs to become involved in this world of discovery. Today, however, formal training and modern laboratory facilities would probably be integral parts of this process.

Anton van Leeuwenhoek (1632-1723) was born in Delft, Holland and was sent as a boy to Amsterdam to be trained for business. Returning to Delft, he opened a drapery shop and was later appointed to a position in the city government. In his spare time he pursued his hobby of constructing his own microscopes and examining many different materials with them.

Using lenses which he ground himself, Leeuwenhoek improved upon the single lens microscope. He made more than 400 lenses, some of them having a magnification of more than 250 times. Although his instruments were in great demand he never sold them but sometimes gave some as gifts.

As a research worker Leeuwenhoek was a self-taught man, never

having received any formal scientific training. He took careful notes of all that he observed and sent them to the Royal Society of London in the form of letters.

Leeuwenhoek's contribution to the development of biological science was as an observer rather than as a solver of problems. He coined no new scientific terms, attempted no classifications, and wrote his letters in a non-scientific style. Yet, his wanderings through the natural world with the microscope provided vital information for future generations of scientific investigators.

The following paper illustrates Leeuwenhoek's style of investigation and his manner of describing his observations.

OBSERVATIONS . . . CONCERNING LITTLE ANIMALS, etc.

ANTON van LEEUWENHOEK (1677)

In the year 1675, about half-way through September (being busy with studying air, when I had much compressed it by means of water), I discovered living creatures in rain, which had stood but a few days in a new tub that was painted blue within. This observation provoked me to investigate this water more narrowly; and especially because these little animals were, to my eye, more than 10,000 times smaller than the animal which Swammerdam has portrayed, and called by the name of waterflea or water-louse, which you can see alive and moving in water with the bare eye.

On August the 2nd, at about 7 o'clock in the evening, I again examined my well-water, which was very clear, especially when it stood in a kettle or pot; but standing in a clean glass, by the side of clear rain-water, the rain-water surpassed the well-water in clearness. In this well-water I saw living a great number of the before-mentioned minute animalcules; some thousands, indeed, in a drop of water.

In the afternoon of the same day, about 3 o'clock, I saw still more animalcules, both round ones and those that were twice as long as broad, and besides these, a sort which were still smaller, and incredibly many of the very little animalcules whose shape I could not make out this morning. I now saw very distinctly that these were little eels or worms, lying huddled together and wriggling, just as if you saw with your naked eye a whole tubful of very little eels and water, the eels moving about in swarms; and the whole water seemed to be alive with the multitudinous animalcules. For me this was among all the marvels that I have discovered in nature the most marvelous of all, and I must say that, for my part, no more pleasant sight has yet met my eye than this of so many thousands of living creatures in one small drop of

water, all huddling and moving, but each creature having its own motion. And even if I said that there were a hundred thousand animalcules in one drop of water which I took from the surface of the water, I should not err. Others, seeing this, would estimate the number of quite ten times more, of which I have instances, but I give the lowest numbers.

These observations concerning living creatures in the above-mentioned liquids, require indeed closer attention and description, but that would also require a whole man which my circumstances do not allow of, and I have employed only my spare time upon them.

In Philosophical Transactions of the Royal Society
Vol. 11. London. 1677. P. 821.

Guide to Paper by Anton van Leeuwenhoek

1. *What indication is there that Leeuwenhoek was extremely curious?*
2. *How does the work of Leeuwenhoek demonstrate that "very great discoveries . . . were so close to the surface that they could be unearthed with little expense by non-conformists with an inquisitive mind, with knowledge and the urge to create?"*
3. *Describe Leeuwenhoek's reactions as he made observations through his microscope.*

Robert Brown—Keen Observation Helps in Solving the Mystery of the Cell

More than one hundred and fifty years after Leeuwenhoek's observations with his microscope and Robert Hooke's description of the cell, Robert Brown (1773-1858) continued the search for new discoveries within the microscopic world. The son of a Scottish clergyman, Brown studied medicine, but after five years as a doctor he was drawn into a new adventure.

British fleets were constantly exploring newly discovered parts of the world. In 1801, an expedition was commissioned to survey the unknown coasts of Australia. Previous voyages had uncovered many plants growing in Australia which were unknown to Europeans. The scientific adviser to King George III recommended that newly found plants from all over the world be observed and tested to see if they could be of any use to the British.

Robert Brown was appointed as chief naturalist to the

Australian voyage of 1801. He and his assistants were given equipment and supplies for their work, but were given no specific instructions. Brown was able to make his own decisions about the biological investigations, although it was understood that the main purpose of the trip was exploration.

In Australia he collected and studied many different kinds of plants (about 4,000 species). When he returned to England in 1805, he took a position with the British Museum, and he became recognized as the leading botanist of his time.

Brown studied plants with great care and made keen observations of their properties. He was noted for his industry, thoroughness, and his ability to see relationships. He also discovered an interesting motion of tiny particles which is known as Brownian movement. In examining plants under the microscope, Brown saw that the plant cells contained smaller structures. He published the results of his observations, thereby attracting the attention of other investigators to the tiny part of the cell which he called the nucleus. Even today the nucleus and its contents are a major area of active research.

THE PRESENCE OF A NUCLEUS IS REPORTED IN THE CELLS OF A NUMBER OF PLANTS

ROBERT BROWN (1833)

I shall conclude my observations on Orchids with a notice of some points of their general structure, which chiefly relate to the cellular tissues.

In each cell of the epidermis of a great part of this family a single circular areola, generally somewhat more opaque than the membrane of the cell, is observable. This areola, which is more or less distinctly granular, is slightly convex. There is no regularity as to its place in the cell; it is not unfrequently however central or nearly so.

Only one areola belongs to each cell. This areola, or nucleus of the cell as perhaps it might be termed, is not confined to the epidermis, being also found not only in the surface but in many cases in the internal cells of the tissue.

In the compressed cells of the epidermis the nucleus is in a corresponding degree flattened; but in the internal tissues it is often nearly spherical, more or less firmly adhering to one of the walls, and projecting into the cavity of the cell.

The nucleus is found also in the tissue of the stigma, where it has an

intermediate form, being neither so much flattened as in the epidermis, nor so convex as it is in the internal tissue of the column.

The nucleus of the cell is not confined to Orchids, but is equally present in many other Monocotyledonous families; and I have even found it in the epidermis of Dicotyledonous plants.

In Transactions of the Linnean Society. Vol. 16. No. 2. London. 1833. Pp. 710-713.

Guide to Paper by Robert Brown

1. *How can one explain the fact that "Robert Brown . . . first realized the importance of a phenomenon which, though previously observed, had remained neglected?"*
2. *List four characteristics of the nucleus described by Brown that you have either observed or come across in reading the text or class notes.*
3. *Which characteristics of the scientist are most exemplified by Brown's work?*
4. *Compare the contributions of Hooke, Leeuwenhoek, and Brown to the development of biological thought.*

BIOLOGY OF MAN

William Harvey—The Triumph of Experimental Methods Over Ancient Beliefs

The discovery of the circulation of the blood by William Harvey (1578-1657) marks the establishment of the experimental method in biology. He was the first biologist to use quantitative methods such as weighing, measuring, and counting to demonstrate an important discovery.

Born in England, Harvey studied medicine at the University of Padua in Italy, the greatest scientific school of its time. He was taught the theory of the ancient Greek physician Galen concerning the movement of blood in the body. According to Galen, a special substance passed from the intestines to the liver which converted this substance into the blood which flows through veins.

After returning to England, Harvey began his medical career and treated the famous philosopher Francis Bacon as one of his patients. He later became physician to King James I of England. In 1616—the year in which Shakespeare died—Harvey was lecturing at the Royal College of Physicians where he had delivered his paper on how the heart acts and how the blood moves. He did not publish his complete theory until 1628, as it was necessary to provide enough experimental evidence to support it. In order to gather this information, Harvey dissected forty different species of animals.

A few years after publishing his theory, he was appointed physician to King Charles I. Harvey attended the King on the battlefield during the civil war. Because of this his house was broken into, and his manuscripts and collections were destroyed by opponents of the king.

After the end of the war, Harvey lived in retirement in London. It took between thirty and fifty years for his theory on circulation to be generally accepted. After this acceptance, further advances in biology followed very rapidly.

ANATOMICAL DISSERTATION CONCERNING THE MOTION OF THE HEART AND BLOOD IN ANIMALS

WILLIAM HARVEY (1628)

So far we have considered the transfer of blood from the veins to the arteries, and the ways by which it is transmitted and distributed by the heart beat. There may be some who will agree with me on these points because of the authority of Galen or Columbus or the reasons of others. What remains to be said on the quantity and source of this transferred blood, is even if carefully reflected upon, so strange and undreamed of, that not only do I fear danger to myself from the malice of a few, but I dread lest I have all men as enemies, so much does habit or doctrine once absorbed, driving deeply its roots, become second nature, and so much does reverence for antiquity influence all men. But now the die is cast; my hope is in the love of truth and in the integrity of intelligence.

First I seriously considered in many investigations how much blood might be lost from cutting the arteries in animal experiments. Then I reflected on the symmetry and size of the vessels entering and leaving the ventricles of the heart, for Nature, making nothing in vain, would not have given these vessels such relative greatness uselessly. Then I thought of the arrangement and structure of the valves and the rest of the heart. On these and other such matters I pondered often and deeply. For a long time I turned over in my mind such questions, as how much blood is transmitted, and how short a time does its passage take. Not deeming it possible for the digested food mass to furnish such an abundance of blood, without totally draining the veins or rupturing the arteries, unless it somehow got back to the veins from the arteries and returned to the right ventricle of the heart, I began to think there was a sort of MOTION AS IN A CIRCLE.

This I afterwards found true, that blood is pushed by the beat of the left ventricle and distributed through the arteries to the whole body, and back through the veins to the vena cava, and then returned to the right auricle, just as it is sent to the lungs through the pulmonary artery from the right ventricle and returned from the lungs through the pulmonary vein to the left ventricle, as previously described.

This motion may be called circular in the way that Aristotle says air and rain follow the circular motion of the stars. The moist earth warmed by the sun gives off vapors, which, rising, are condensed to fall again moistening the earth. By this means things grow. So also tempest and meteors originate by a circular approach and recession of the sun.

Thus it happens in the body by the movement of the blood, all parts are fed and warmed by the more perfect, more spiritous, hotter, and I might say, more nutritive blood. But in these parts this blood is cooled, thickened, and

loses its power, so that it returns to its source, the heart, the inner temple of the body, to recover its virtue.

Here it regains its natural heat and fluidity, its power and vitality, and filled with spirits, is distributed again. All this depends on the motion and beat of the heart.

If anyone says these are empty words, broad assertions without basis, or innovations without just cause, there are three points coming for proof, from which I believe the truth will necessarily follow, and be clearly evident.

First, blood is constantly being transmitted from the vena cava to the arteries by the heart beat in such amounts that it cannot be furnished by the food consumed, and in such a way that the total quantity must pass through the heart in a short time.

Second, blood is forced by the pulse in the arteries continually and steadily to every part of the body in a much greater amount than is needed for nutrition or than the whole mass of food could supply.

And likewise third, the veins continually return this blood from every part of the body to the heart.

These proved, I think it will be clear that the blood circulates, passing away from the heart to the extremities and then returning back to the heart, thus moving in a circle.

Works. Robert Willis, Editor. Sydenham Society. London. 1847. Also published by E. P. Dutton. 1906.

Guide to Paper by William Harvey

1. *What did Harvey mean when he said that some people would agree with his views "because of the authority of Galen or Columbus?"*
2. *Why was Harvey afraid of the reaction of people to his views on circulation?*
3. *It was originally thought that blood was constantly being made by the body from food. How did Harvey help to disprove this idea?*
4. *Summarize very briefly and in your own words, Harvey's argument for the circulation of the blood.*
5. *Harvey thought that there were differences in blood before and after it got to the parts of the body. He called blood leaving the heart "more spiritous, hotter, and . . . . more nutritive . . . ." He said that at the parts of the body blood "is cooled, thickened, and loses its power." How could one interpret this today?*
6. *What one element was lacking in Harvey's account of the circulation?*
7. *Why do you think Harvey was unable to discover the above element?*

Marcello Malpighi—How the Study of a Frog Helped Extend the Work of Harvey

An analysis of William Harvey's paper on the circulation of the blood reveals that Harvey could not explain how blood passed from the arteries into the veins. In the year that Harvey published this work in England, Marcello Malpighi (1628-1694) was born in Italy, and it remained for him to complete Harvey's investigations.

Malpighi attended the University of Bologna until the death of his parents forced him to postpone his education in order to care for his younger brothers and sisters. He completed his studies in medicine in 1653 and then devoted himself to medical practice and teaching. At this time, a dispute involving the boundary lines between his father's property and the adjoining land of another family created a feud which lasted throughout his life. He was subjected to attacks by this family who attempted to injure both his scientific reputation and his name. However, Malpighi faced these assaults on his character with dignity.

He was among the first scientists to use microscopes in studying animal and plant structure. As microscope making improved in the 30 years after Harvey's discovery, Malpighi then was able to see what was invisible to Harvey. In this paper Malpighi relates what he saw through the microscope.

In the career of Marcello Malpighi we see the beginnings of microscopic anatomy. In addition to completing the work of Harvey, Malpighi also made significant discoveries in the areas of respiration and in the structure of glands. Parts of the skin, the kidney, and the insect body are named after him. His career was climaxed when he was appointed personal physician to Pope Innocent XII in 1691.

ON THE LUNGS

MARCELLO MALPIGHI (1661)

I set out to become more certain about the membranous substance of the lungs by studying the anatomy of frogs. It happened to me to see such things that can best be described by the saying of Homer–"I see with my eyes a work trusty and great!"

Observations by means of the microscope will reveal more wonderful things than those viewed in regard to mere structure and connection. While the heart is still beating, the movement of the blood is observed in the vessels—though with difficulty—so that the circulation of the blood is clearly exposed. This is more clearly recognized in the mesentery and in the other great veins contained in the abdomen.

Thus by this impulse the blood is driven in very small streams through the arteries like a flood into the several cells, one or another branch clearly passing through or ending there. Thus blood, much divided, cuts off its red colour, and, carried round in a winding way is poured out on all sides till at length it may reach the walls, the angles, and the absorbing branches of the veins.

The power of the eye could not be extended further in the opened living animal, hence I had believed that this body of the blood breaks into the empty space, and is collected again by a gaping vessel and by the structure of the walls. But the dried lung of the frog made my belief dubious. This lung had, by chance, preserved the redness of the blood in what proved to be the smallest vessels. And, so great is the branching of these vessels as they go out, here from a vein, there from an artery, that order is no longer preserved, but a network appears made up of the prolongations of both vessels. This network occupies not only the whole floor, but extends also to the walls, and is attached to the outgoing vessel, as I could see with greater difficulty but more abundantly in the oblong lung of a tortoise, which is similarly membranous and transparent. Here it was clear to sense that the blood flows away through the tortuous vessels, that it is not poured into spaces but always works through tubules, and is dispersed by the multiple winding of the vessels, since the same holds in the intestines and other parts; nay, what seems more wonderful, she joins the upper and the lower ends of veins to one another by visible connections.

But in order that you may more easily get hold of what I have said, and follow it with your own sight, tie with a thread, just where it joins the heart, the projecting swollen lung of an opened frog while it is bathed on every side with abundant blood. You will see this very well if you examine it by the microscope of one lens against the horizontal sun. Or you may institute another method of seeing these things. Place the lung on a crystal plate illuminated below through a tube by a lighted candle. To it bring a microscope of two lenses, and thus the vessels distributed in a ring-like fashion will be disclosed to you. By the same arrangement of the instrument and light, you will observe the movement of the blood through the vessels in question. You will yourself be able to contrive it by different degrees of light, which escape description by the pen.

From these things, therefore, as to the first problems to be solved, from analogy and the simplicity which nature uses in all her operations, it can be

inferred that the network which formerly I believed to be nervous in nature, mingled in the bladder and sinuses, is really a vessel carrying the body of the blood thither or carrying it away. Also that, although in the lungs of perfect animals the vessels seem sometimes to gape and end in the midst of the network of rings, nevertheless, it is likely that, as in the cells of frogs and tortoise, that vessel is prolonged further into small vessels in the form of a network, and these escape the senses on account of their exquisite smallness.

In Proceedings of the Royal Society of Medicine. Vol. 23. James Young, Translator. London. 1929. Also "Opera Omnia," Robert Littlebury edition. London, 1687.

Guide to Paper by Marcello Malpighi

1. *What was Malpighi's first belief about the path of blood before he used the microscope?*
2. *Why did he change this belief?*
3. *How did the work of Malpighi complete the studies of Harvey?*
4. *Why was Malpighi able to accomplish this (above) while Harvey could not?*
5. *Explain the following statement: "Harvey made the existence of capillaries a logical necessity; Malpighi made it a certainty."*

Anton van Leeuwenhoek—How a Hobby Led to the Completion of Harvey's Work on Circulation

Although Harvey was able to demonstrate that the blood circulates, he did not observe the final links in the circulatory chain. The capillaries, which transmit the blood from the arteries to the veins, were discovered by Malpighi four years after Harvey's death. Yet, further investigations were needed to explain and to complete the knowledge of the capillary system which Malpighi originated.

We have seen how Anton van Leeuwenhoek (1632-1723), the amateur scientist, pursued his hobby of making microscopes and peering into the invisible world. In 1686 he saw the fine circulation of the blood. His observations, coming 25 years after Malpighi's work, helped to confirm the truth of Harvey's theory on circulation and added more details than Malpighi provided.

Leeuwenhoek noted that the blood cells of birds, frogs, and fish

were oval in shape and not like globules of fat as had been believed previously. He also found that human blood cells are circular. He made many other observations which aided medicine and physiology.

Harvey, Malpighi, and Leeuwenhoek all were alive at the same time. Harvey was physician to King Charles I of England and Malpighi was physician to a pope. Leeuwenhoek's achievements brought him no similar honors, yet all shared some of the excitement of an age of exploration and artistic progress. Navigators from many countries extended man's knowledge of different parts of the earth. As a new nation was being formed in the American colonies, European masters, such as the playwright Shakespeare, the painter Rembrandt, and the scientist Galileo, were helping to shape Western thought for many years to come. It was during this time span—from the end of the 1500s to the end of the 1600s that Harvey, Malpighi, and Leeuwenhoek helped people understand how blood circulates in the human body.

ON THE CAPILLARY CIRCULATION

ANTON van LEEUWENHOEK (1632-1723)

Herewith I again send you some of my trifling observations.

I was very greatly pleased to see very distinctly the circulation of the blood in the tadpole, which was driven on from the parts that were nearest to the body to those on the outside, thus causing an uninterrupted, very rapid circulation.

In another place I saw that three of the thinnest arteries, each running in a curve, all met together in one point and there formed a blood vessel or vein, and consequently this blood vessel was as wide as the three arteries mentioned. These three distinct vessels with their somewhat circular course, in which the circulation took place were so small that a grain of sand would have covered them.

Such blood vessels running across each other I often noticed before when I tried to discover the junction of arteries and veins in other animals, but I was quite certain that the return circuit of the blood does not take place in the large vessels, but in the smallest or thinnest, for if it were otherwise, I concluded that all the parts of the body could not be fed. And as these discoveries seemed inscrutable to me, I gave up my investigations on this head for some years. If now we see clearly with our eyes that the passing of the

blood from the arteries into the veins, in the tadpoles, only takes place in such blood vessels as are so thin that only one corpuscle can be driven through at one time, we may conclude that the same thing takes place in the same way in our bodies as well as in that of all animals.

I have said before that the corpuscles or globules that make the blood red, are so small that ten hundred thousand of them are not so big as a grain of coarse sand, and so we easily imagine how very small the blood vessels are in which the circulation of blood takes place.

The observations told here have not been made once, but they have been resumed repeatedly, giving me much pleasure, and every time on different tadpoles, and the result has almost always been the same.

When I looked along the length of the tail and at the thickest part of it, I could clearly see that, on either side of the bone there was a large artery, through which the blood was carried to the extremity of the tail, and which on its way sent out several small branches.

When I looked at the part of the tail beside these arteries on the outside, I discovered there two large veins, which carried the blood back again to the heart, and moreover I saw that blood was driven into this large vein from several small veins. In short, I saw here the circulation of the blood to my perfect satisfaction, because there was nothing, though ever so slight, that caused me any doubt.

Also I observed the young frogs when they had changed from tadpoles into frogs and I also discovered in them a very large number of small blood vessels which, continually running in curves formed the vessels called arteries and veins, from which it was perfectly clear to me that the arteries and veins are one and the same continuous blood vessels. But I saw them clearest of all and most of all at the end of the projecting parts of the legs, which we may call fingers, and of which the frog has four on each fore-leg and five on each hind-leg.

In Leeuwenhoek and His Little Animals, Quarto 7. Clifford Dobell, Collector, Translator, and Editor. Harcourt, Brace, Jovanovich, Inc. New York. 1932.

Guide to Paper by Anton van Leeuwenhoek

1. *How did Leeuwenhoek extend the knowledge of the capillary circulation which Malpighi had discovered?*
2. *a. Where did Leeuwenhoek observe capillary circulation? b. Why was the above a very suitable choice for this purpose?*
3. *Why might it be said that Malpighi observed capillaries while Leeuwenhoek observed capillary circulation?*

4. *Is there any indication that Leeuwenhoek either was a keener observer than Malpighi or that he had more sensitive microscopes?*
5. *What attempt did Leeuwenhoek make to assure the reliability of his observations?*
6. *With discoveries such as those by Harvey, Malpighi, and Leeuwenhoek illuminating the mechanism of the movement of blood, which aspect of circulation do you think was next open for scientific investigation?*

William Beaumont—A Scientific Discovery from the Early American Frontier

It is probably difficult for one living in the United States today to picture what it was like in this country about thirty years after the inauguration of President George Washington. Vast areas of the nation were nothing more than wilderness. Forts occupied by United States troops were present to aid in settling the frontier. Fort Mackinac, located on the Great Lakes in Michigan, was a meeting place for Indians and travelers involved in the fur trade.

William Beaumont (1785-1853), the son of a Connecticut farmer, was serving as an army surgeon at this fort. In 1822 a rare opportunity to investigate a function of the human body presented itself to Dr. Beaumont. In medical research up to this time, there were very few reports or direct observations of what happens within the living human body. Beaumont provided the world with such information as a result of his 11-year study of Alexis St. Martin. He did this work despite the difficulty of maintaining continuous direct contact with his patient. It was often necessary for Beaumont to follow St. Martin as he moved from place to place.

In the work of William Beaumont one sees how it is possible for scientific discovery to occur far from established laboratories and even under frontier conditions.

EXPERIMENTS AND OBSERVATIONS ON THE GASTRIC JUICE AND THE PHYSIOLOGY OF DIGESTION

WILLIAM BEAUMONT (1833)

Alexis St. Martin, who is the subject of these experiments, was a Canadian, of French descent, at the above mentioned time about eighteen years of age,

of good constitution, robust and healthy. He had been engaged in the service of the American Fur Company, as a voyageur, and was accidentally wounded by the discharge of a musket, on the 6th of June, 1822.

I saw him in 25 or 30 minutes after the accident occurred, and, on examination, found a portion of the lung protruding through the external wound, and immediately below this, another protrusion, which on further examination, proved to be a portion of the stomach, lacerated through all its coats, and pouring out the food he had taken for his breakfast, through an orifice large enough to admit the fore finger.

One year from the time of the accident, a small fold or doubling of the coats of the stomach appeared, forming at the superior margin of the orifice, slightly protruding, and increasing till it filled the aperture, so as to supersede the necessity for the compress and bandage for retaining the contents of the stomach. This valvular formation adapted itself to the accidental orifice, so as to completely prevent the efflux of the gastric contents when the stomach was full, but was easily depressed with the finger.

In the month of May, 1825, I commenced my first series of gastric experiments with him at Fort Mackinac, Michigan Territory.

At 11 o'clock, A.M., after having kept the lad fasting for 17 hours, I introduced the glass tube of a Thermometer (Fahrenheit's) through the perforation, into the stomach, nearly the whole length of the stem, to ascertain the natural warmth of the stomach. In 15 minutes, or less, the mercury rose to 100°, and there remained stationary.

I now introduced a tube, and drew off one ounce of pure gastric juice, unmixed with any other matter, except a small proportion of mucus, into a three ounce vial. I then took a solid piece of boiled, recently salted beef, weighing three drachms, and put it into the juice in the vial: corked the vial tight, and placed it in a saucepan, filled with water, raised to the temperature of 100°, and kept at that point. In forty minutes digestion had distinctly commenced over the surface of the meat. In fifty minutes the fluid had become quite opaque and cloudy; the external texture began to separate and become loose. In sixty minutes, chyme began to form.

At 1 o'clock, P.M., (digestion having progressed with the same regularity as in the last half hour) the cellular texture seemed to be entirely destroyed, leaving the muscular fibers loose and unconnected, floating about in fine small shreds, very tender and soft.

At 3 o'clock, the muscular fibers had diminished one half, since last examination, at 1 o'clock.

At 5 o'clock, they were nearly all digested; a few fibers only remaining.

At 7 o'clock, the muscular texture was completely broken down; and only a few of the small fibers floating in the fluid.

At 9 o'clock, every part of the meat was completely digested.

The gastric juice, when taken from the stomach, was as clear and

transparent as water. The mixture in the vial was now about the color of whey. After standing at rest a few minutes, a fine sediment, of the color of the meat, subsided to the bottom of the vial.

At the same time that I commenced the foregoing experiment, I suspended a piece of beef, exactly similar to that in the vial, into the stomach, through the aperture.

At 12 o'clock, I withdrew it, and found it about as much affected by digestion as that in the vial; there was little or no difference in their appearance. Returned it again.

At 1 o'clock, P.M., I drew out the string; but the meat was all completely digested and gone.

The effect of the gastric juice on the piece of meat, suspended in the stomach, was exactly similar to that in the vial, only more rapid after the first half hour, and sooner completed.

Experiments and Observations on the Gastric Juice and the Physiology of Digestion. Dover Publications, New York. 1959. Originally published 1833.

Guide to Paper by William Beaumont

1. *Why was Alexis St. Martin a suitable subject for an experiment on how digestion takes place in the stomach?*
2. *Why was it possible for Beaumont to continue his observations on St. Martin's condition for many years?*
3. *Why did Beaumont keep the temperature of the vial containing the gastric juice and beef at 100° F?*
4. *What is the present day explanation for the reaction that Beaumont observed in the vial?*
5. *Why did Beaumont conduct an additional experiment which involved suspending a piece of beef directly into the stomach?*

Claude Bernard—The Theater's Loss Is Physiology's Gain

Claude Bernard (1813-1878) was born in the small French village of Saint-Julian in 1813. He left college to work as a druggist's assistant, but his main interest was to write plays for the theater. As a result of the success of one of his plays, he went to Paris when he was 21 to further his career as a playwright. Bernard brought his new play to a leading critic who recommended that Bernard study medicine.

After completing his medical training, Bernard became a professor of physiology at the Sorbonne, where he earned a reputation as a great experimenter. He was admired by the Emperor Napoleon III, who provided him with two excellent laboratories. Prior to Bernard's work, it was thought that the body could be regarded as a bundle of organs, each with its own separate functions. Through painstaking research, Bernard was able to gather sufficient experimental evidence to prove that the various organs of the body worked in harmony and that all were interrelated.

Among his most significant contributions to physiology was his discovery of the part played by the liver in metabolism. Bernard characterized the liver as a "vital laboratory." Through his investigations of the liver, Bernard was able to show how the body can break down and also build up complex chemical substances.

Bernard's career in science illustrates how a person born of poor peasant parents can attain national prominence. In recognition of his many achievements in physiology, Claude Bernard was the first man of science to receive a state funeral when he died in 1878.

ON THE MECHANISM OF FORMATION OF SUGAR IN THE LIVER

CLAUDE BERNARD (1855)

The hypotheses prevalent today as to the mechanism of secretion state that the glandular organ itself supplies nothing to the secretion, but that its tissue is restricted to acting by a sort of contact upon the elements of the blood that pass through the glandular organ at the very moment when the secretion takes place. In the particular case of the secretion of sugar in the liver, all the authors assume that the sugar is formed directly in the blood.

The facts which I shall now reveal are calculated to prove, it seems to me, that the glycogenic function of the liver must be understood quite otherwise, and that instead of searching in the blood for the immediate precursor of sugar, it must be sought in the hepatic tissue itself.

The following experiment illuminates this fact; I shall describe it in some detail so that it may easily be repeated. The results are, in my opinion, very important and worthy of the attention of both physiologists and chemists.

I chose an adult dog, vigorous and in good health, which had been fed for

several days exclusively on meat, and I sacrificed it by severing the medulla seven hours after an ample meal of tripe. The abdomen was immediately opened; the liver was removed avoiding injury to its tissue, and the organ, while still warm and before the blood had had time to coagulate in its vessels, was washed with cold water through the portal vein. Under the influence of this energetic washing, the liver swelled, the color of its tissue became pale, and the blood was expelled with the water which escaped in a strong continuous jet through the hepatic veins. At the end of a quarter of an hour the tissue of the liver was already nearly bloodless, and the water which emerged from the hepatic veins was entirely colorless. I subjected the liver to this continuous washing for 40 minutes without interruption. I had determined at the beginning of the experiment that the red colored water which flowed out of the hepatic veins was sweet and gave an abundant precipitate on heating, and I verified at the end of the treatment that the perfectly colorless water which emerged from the hepatic veins did not contain any traces of sugar.

The liver was then removed from the action of the water current; and I made sure, by boiling a piece of the liver with a little water, that its tissue was well washed, since it no longer contained sugar. I then left this liver in a jar at room temperature. After twenty four hours I found that this organ, washed entirely bloodless, which I had left the night before completely free of sugar, now contained sugar in abundance. I was sufficiently convinced of this when I examined a little of the liquid which had flowed out around the liver and which was very sweet; then by injecting cold water into the portal vein with a small syringe and collecting this water as it escaped through the hepatic veins, I found that this liquid fermented very abundantly and very actively with yeast.

This simple experiment, in which one can see before his eyes the abundant reappearance of sugar in a liver which had been completely deprived of it and of its blood by means of the washing, is most instructive for the solution of the problem of glycogen function. This experiment clearly proves, as we have already said, that in a fresh liver in the physiological, i.e., functional, state there are two substances: (1) sugar, which is very soluble in water and is carried away by the blood during washing; and (2) another substance, so little soluble in water that it remains bound to the hepatic tissue after the latter has been freed of its sugar and its blood by forty minutes' washing.

It is the latter substance which, in the undisturbed liver, gradually changed to sugar by a kind of fermentation, as we shall show.

In Great Experiments in Biology. M. Gabriel and S. Fogel, Editors. Prentice-Hall, Inc., Englewood Cliffs, New Jersey. 1955. From Comptes-Rendus de l'Académie de Sciences. Vol. 41. Paris. 1855. Pp. 461-469.

Guide to Paper by Claude Bernard

1. *What hypothesis was Bernard trying to disprove?*
2. *What was his own hypothesis?*
3. *Briefly describe the method that Bernard used in order to prove his hypothesis.*
4. *What were his observations (to the above method)?*
5. *How did Bernard's observations support his hypothesis?*
6. *What is the name for the substance Bernard describes as "so little soluble in water that it remains bound to the hepatic tissue after the latter has been freed of its sugar . . . .?*
7. *Why did Bernard wash the liver by running cold water through the portal vein?*

William M. Bayliss and Ernest H. Starling— Discovery of the Cause of Pancreatic Secretion

For centuries there had been a vague idea that each organ contributed some substance to the blood. Claude Bernard's paper reporting his work on glycogen focused attention on this belief. However, there was no general agreement among biologists until the research of the two English scientists Bayliss and Starling in 1902.

William Bayliss (1860-1924) and Ernest Starling (1866-1927) were each recognized as the most distinguished physiologists of their generation. Bayliss began his training as a medical student but preferred scientific research to the practice of medicine. He became associated with the physiological laboratories of the University of London where he met Starling. During World War I, Bayliss experimentally investigated the nature of wound-shock and devised methods which were widely and successfully used to save many lives. For these and other accomplishments Bayliss was knighted by King George V in 1922.

Starling was also trained as a physician but he, too, turned his attention to research. He applied physics and chemistry to the solving of physiological problems. In addition to his work with Bayliss, Starling contributed significant knowledge concerning the mechanism of the heart and circulation. Probably no physiologist since Harvey had so greatly advanced an understanding of the

heart's action. During World War I, Starling attempted to develop defenses against poison gas.

Their joint work on the mechanism of pancreatic secretion served to eliminate the previous confusion in this area and introduced the term hormone to biology. Interest in hormones spread widely during the twentieth century and has led to advances in all fields of biology and in all branches of clinical medicine.

THE MECHANISM OF PANCREATIC SECRETION

WILLIAM M. BAYLISS AND ERNEST H. STARLING (1902)

We soon found, however, that we were dealing with an entirely different order of phenomena, and that the secretion of the pancreas is normally called into play not by nervous channels at all, but by a chemical substance which is formed in the mucous membrane of the upper parts of the small intestine under the influence of acid, and is carried thence by the blood stream to the gland-cells of the pancreas.

On January 16, 1902, a dog of about 6 kilos weight, which had been fed about 18 hours previously, was given a hypodermic injection of morphia some 3 hours before the experiment. The nervous masses around the superior mesenteric artery and coeliac axis were completely removed and both vagi cut. A loop of jejunum was tied at both ends and the mesenteric nerves supplying it were carefully dissected out and divided, so that the piece of intestine was connected to the body of the animal merely by its arteries and veins. A cannula was inserted in the large pancreatic duct and the drops of secretion recorded. The blood pressure in the carotid was also recorded in the usual way. The animal was in the warm saline bath and under artificial respiration.

The introduction of 20 c.c. of 0.4% HCl [hydrochloric acid] into the duodenum produced a well-marked secretion of 1 drop every 20 seconds, lasting for some 6 minutes; this result merely confirms previous work.

But, and this is the important point of the experiment, and the turning point of the whole research, the introduction of 10 c.c. of the same acid into the enervated loop of jejunum produced a similar and equally well-marked effect.

Now, since this part of the intestine was completely cut off from the nervous connection with the pancreas, the conclusion was inevitable that the effect was produced by some chemical substance finding its way into the

veins of the loop of jejunum in question and being carried in the blood stream to the pancreatic cells. Wertheimer and Lepage have shown, however, that acid introduced into the circulation has no effect on the pancreatic secretion, so that the body of which we were in search could not be the acid itself. But there is, between the lumen of the gut and the absorbent vessels, a layer of epithelium, whose cells are as we know endowed with numerous important functions. It seemed therefore possible that the action of acid on these cells would produce a body capable of exciting the pancreas to activity. The next step in our experiment was plain; to cut out the loop of jejunum, scrape off the mucous membrane, rub it up with sand and 0.4% HCl in a mortar, filter through cotton-wool to get rid of lumps and sand, and inject the extract into a vein. The first effect is a considerable fall of blood pressure, due as we shall show later, to a body distinct from that acting on the pancreas, and after a latent period of about 70 seconds a flow of pancreatic juice at more than twice the rate produced at the beginning of the experiment by introduction of acid into the duodenum. We have already suggested the name "secretin" for this body, and as it has been accepted and made use of by subsequent workers it is as well to adhere to it.

Journal of Physiology 28:325-353; 1902.

Guide to Paper by William M. Bayliss and Ernest H. Starling

1. *What hypothesis as to the mechanism of pancreatic secretion were the investigators attempting to disprove?*
2. *What was their hypothesis?*
3. *Why were all the nerves around the section of the small intestine being studied cut?*
4. *Why was hydrochloric acid chosen to be introduced into the small intestine?*
5. *Why do the investigators regard the fact that the acid produced exactly the same effect (the secretion of pancreatic juice) in parts of the small intestine with or without nerves as "the turning point of the whole research?"*
6. *Why was the acid itself eliminated as the cause of pancreatic secretion?*
7. *Why was an extract prepared from a section of the small intestine by grinding up the section?*
8. *Why was the above extract injected into the blood of the animals?*

Frederick G. Banting and Charles H. Best—A Major Step on the Road to a Successful Treatment for Diabetes

The disease diabetes mellitus has been known to man since the early days of the Roman Empire. "Diabetes" is a word of Greek origin meaning "to go through" while "mellitus" in Latin means "honey." Therefore the actual meaning of diabetes mellitus is "honey goes through," honey referring to the sugar which passes from the blood into the urine of one who suffers from the condition.

In 1788 an English physician first suggested a relationship between diabetes and the pancreas. One hundred years later, two German physiologists discovered that the removal of this organ from dogs immediately caused them to develop severe diabetes. It was not until 1921 that Frederick Banting (1891-1941), working with his young assistant, Charles Best, completed further investigations into the nature of the relationship between diabetes and the pancreas.

Frederick Banting was a Canadian scientist who turned to a career in research after practicing medicine for several years. He worked at the University of Toronto, where together with Professor J. R. McLeod and the young medical student Charles Best, he conducted experiments involving the pancreas. Banting was 31 years old when the team made its important discovery. Their efforts were rewarded with the Nobel Prize for medicine in 1923. Banting continued his investigations after receiving this award, in research foundations and institutes set up for him by the Canadian Government. His career was ended when he was killed in an airplane accident at the age of 50.

The following paper describes some of the methods used by Banting and his co-workers. Continuation of their research by other investigators has resulted in the successful treatment of diabetes by modern medicine.

THE INTERNAL SECRETION OF THE PANCREAS

FREDERICK G. BANTING AND CHARLES H. BEST (1922)

We have found that animals between eight and sixteen months old are most suitable for this operation (removal of the pancreas). At this age the pancreas is not so firmly fixed as it becomes later.

The extract was prepared as follows: The dog was given a lethal dose of chloroform. The degenerated pancreas was swiftly removed and sliced into a chilled mortar containing Ringer's solution. The mortar was placed in freezing mixture and the contents partially frozen. The half frozen gland was then completely macerated. The solution was filtered through paper and the filtrate, having been raised to body temperature, was injected intravenously.

We performed several experiments with the object of exhausting the zymogen granules of the pancreas. Prolonged secretin injections and vagus stimulation below the diaphragm were practiced. Fortune favored us in the first experiment. In subsequent attempts we were never able to exhaust the gland sufficiently to obtain an extract free from the disturbing effects of some constituent of pancreatic juice.

At 6 P.M. September 8, we administered 10 c.c. of extract of degenerated pancreas. There was no reduction in blood sugar at 7 P.M. when we gave 12 c.c. of extract of exhausted gland intravenously. The blood sugar rapidly fell from .30% to .21%; subsequent injections of the same material produced a further drop in blood sugar to .07%. At 6 A.M., September 10th the blood sugar having returned to .27% we administered 15 c.c. of extract of exhausted gland. There was no effect. At 8 A.M., September 10, 15 c.c. of extract of exhausted gland were injected intravenously. The drop in blood sugar to .13% was very marked.

A short, but very interesting experiment again demonstrates the remarkable effect of the extract of degenerated pancreas upon the power of a diabetic animal to retain sugar. On November 8 at 11 A.M. (Blood sugar .35%), 10 grams of sugar were injected intravenously. In one hour the blood sugar rose to .40%. In the four hours following the injection, 10.8 grams of sugar were excreted. From 3 to 9 P.M. 78 c.c. of dilute extract were injected in 13 c.c. doses. The blood sugar fell to .09%. At 9 P.M. (blood sugar at .09%), 10 grams of sugar were injected. At 10 P.M. the blood sugar had risen to .22%.

In the course of our experiments we have administered over seventy-five doses of extract from degenerated pancreatic tissue to ten different diabetic animals. Since the extract has always produced a reduction of the percentage sugar of the blood and of the sugar excreted in the urine, we feel justified in stating that this extract contains the internal secretion of the pancreas. Some of our more recent experiments, which are not yet completed, give, in

addition to still more conclusive evidence regarding the sugar retaining power of diabetic animals treated with extract, some interesting facts regarding the chemical nature of the active principle of the internal secretion. These results, together with a study of the respiratory exchange in diabetic animals before and after administration of extract, will be reported in a subsequent communication.

We have always observed a distinct improvement in the clinical condition of diabetic dogs after administration of extract of degenerated pancreas, but it is very obvious that the results of our experimental work, as reported in this paper, do not at present justify the therapeutic administration of degenerated gland extracts to cases of diabetes in the clinic.

Journal of Laboratory and Clinical Medicine
7:251-256; February 1922.

Guide to Paper by Frederick G. Banting and Charles H. Best

1. *What were Banting and Best attempting to do?*
2. *The substance that Banting and Best were interested in was later found to be a protein. How did this add to the difficulty of what the investigators were trying to do?*
3. *Why was it necessary to give "prolonged secretin injections" before preparing the extract?*
4. *What was the effect of injections of the extract prepared from the pancreas?*
5. *What name is given today to the substance the investigators call "the internal secretion of the pancreas?"*
6. *What indication is there that the investigators were cautious in applying the results of their experiments?*

Christiaan Eijkman—A Disrupted Experiment Leads to a Cure for Beriberi

Christiaan Eijkman (1858-1930) was born in The Netherlands and studied medicine at the University of Amsterdam. In 1895 he was appointed as a member of a commission sent by the Dutch Government to its East Indian Colonies in order to investigate a disease which was crippling the native population. This disease, called beriberi, was widespread throughout Asia. The name is an Asian one meaning "extreme weakness."

At this time Pasteur's idea that many diseases were caused by microorganisms was gaining great popularity. Therefore, it was natural for Eijkman's commission to search for the germ responsible for beriberi. The commission spent two years on this task without any success.

One day Eijkman noticed that chickens in a hospital yard showed the same signs of weakness as did the beriberi patients. He found that these chickens were fed leftover food from dinners served to the patients. The main item in the native diet was white, polished rice (rice with the husk or outer covering removed). Eijkman's hypothesis was that a germ must have been transmitted from the patients to the chickens through the leftover food. He designed an experiment to test this hypothesis. The hospital superintendent, objecting to the use of costly, polished rice as chicken feed in the experiment, substituted cheaper, unpolished rice without consulting Eijkman. The Dutch investigator was disturbed by the disruption of his experiment until he observed a remarkable recovery among the chickens receiving the unpolished rice. This observation led to a new hypothesis and new experiments regarding the cause of beriberi. In order to test this hypothesis among humans, Eijkman compared groups of people who suffered from beriberi with those who did not.

The accompanying paper relates Eijkman's survey of beriberi among prisoners of the Dutch East Indies.

AN ATTEMPT TO COMBAT BERIBERI

CHRISTIAAN EIJKMAN (1897)

Since last year research has been carried out on the island of Java with regard to a possible connection between the main dietary component and the incidence of beriberi in the native prisons. These tests were initiated by the results of my studies on a chicken disease similar to beriberi. I was able to establish that the disease is caused by feeding certain grains, especially rice. Only polished rice (raw or boiled) proved to be harmful; unpolished rice was tolerated quite well by the chickens and the same is true of half-polished rice, i.e., with the grains freed from the hulls, but still covered by the cuticles. From these experiments I drew the conclusion that the cuticles probably contain a substance or substances which neutralize the harmful influence of the starchy nutriment.

As supervisor of the Civil Health Department of Java, Herr Vorderman knew that according to local customs, in some parts polished rice, in other parts half-polished rice was given to the native prisoners as a main diet. Therefore it was ascertainable whether there existed a connection between the type of diet and the occurrence of beriberi in the prisons. The reports received covered about 100 prisons on Java and on the small neighboring island of Madura, a number which seems to be sufficient to obtain accurate statistics.

On investigation of the rice samples it was found that the distinction between polished and "half-polished" rice was not sufficient because in not a few cases the grains were only partly freed of the cuticles. The rice samples were therefore classified into three groups:

(1) Half-polished rice: The cuticles entirely or at least 75% preserved.
(2) Mixture of 1 and 3.
(3) Polished rice: The cuticles entirely or at least 75% removed.

TABLE I

It was found that beriberi occurs:

1. (half-polished rice) in one out of 35 prisons, i.e.,	2.7%
2. (mixture) in 6 out of 13 prisons, i.e.,	46.1%
3. (polished rice) in 36 out of 51 prisons, i.e.,	70.6%

The influence of the kind of rice diet shows up even more clearly than in the first inquiry. In full agreement with my observations on the chicken disease, the results are more favorable the more the bran material (cuticle) has been preserved. This is true also of the disease incidence. In the single prison of group 1 in which beriberi cases had occurred, the number of cases was only 0.16%. In the second group, somewhat higher numbers were found, though all of them were still less than 1%. Finally, in the third group, the morbidity in two-thirds of the prisons affected by beriberi was greater than 1%, often over 10%, and in one case it reached the extremely high number of 37%.

TABLE II

The average number of those affected by beriberi was:

In group 1: 1 out of 10,000 prisoners
In group 2: 1 out of 416 prisoners
In group 3: 1 out of 39 prisoners
These statistics cover a total of 279,629 prisoners.

Those who have looked for the cause of beriberi in the rice diet have attributed harmful effects to old rice, more or less spoiled by long storage. In the Dutch Indies imported rice had been mostly blamed. But my experiments on chickens showed the same results no matter what the variety or place of origin of the rice. The disease could be caused equally with polished foreign

rice or polished Java rice. And the fact that the chicken disease was not caused by faulty preservation of the rice followed from the fact that the disease also appeared when the rice was fed immediately after polishing.

To what extent some other hygienically important factors have an influence can be seen from the following data:

TABLE III

Age of Buildings	
40-100 years	beriberi in 13 out of 26 prisons, i.e., in 50.0%
21-40 years	beriberi in 11 out of 32 prisons, i.e., in 34.7%
2-20 years	beriberi in 19 out of 42 prisons, i.e., in 45.2%
Ventilation	
good	beriberi in 28 out of 68 prisons, i.e., in 41.2%
medium	beriberi in 8 out of 11 prisons, i.e., in 72.7%
faulty	beriberi in 7 out of 21 prisons, i.e., in 33.3%
Population density	
sparsely populated	beriberi in 32 out of 73 prisons, i.e., in 44.6%
medium	beriberi in 1 out of 1 prisons, i.e., in 100.0%
overcrowded	beriberi in 9 out of 26 prisons, i.e., in 34.6%

In no case are the differences so great and so marked as to warrant drawing even a somewhat positive conclusion.

Virchow's Archives. Vol. 149. Pp. 187-194, as translated by M. Gabriel and S. Fogel in Great Experiments in Biology, Prentice-Hall, Inc., Englewood Cliffs, New Jersey. 1955.

Guide to Paper by Christiaan Eijkman

1. *What was Eijkman's hypothesis as to the cause of beriberi?*
2. *What hypothesis or hypotheses was Eijkman presenting evidence against?*
3. *What do the data in Table I and in Table II indicate?*
4. *What other possible causes of beriberi were considered by Eijkman?*
5. *What do the data in Table III indicate?*
6. *What is the modern explanation of the cause of beriberi?*

BIOLOGY OF MAN–SUMMARY QUESTIONS

1. *Compare the methods used by each of the investigators in making their discoveries.*
2. *What was the significance of each discovery to our understanding of how the human body functions?*
3. *Select one of the investigators and prepare a report about the times in which he lived. Include major historical events, types of governments in power, forms of communication, methods and rates of travel, kinds of housing and clothing.*

BIOLOGY OF PLANTS

Jean Baptiste van Helmont—From Alchemy to Plant Research

Jean Baptiste van Helmont (1577-1644) was a Belgian physician who spent his life investigating chemical processes. He was a man of extreme contrasts. Deeply religious with strong leanings to the mystical and supernatural, Van Helmont was greatly influenced by the work of scientists such as Harvey and Galileo and was himself a careful observer and an exact experimenter.

After practicing medicine in Brussels, Van Helmont married an heiress, moved to the countryside and there was able to devote his time to research.

He attempted to use chemical knowledge to understand such functions of the body as digestion and nutrition. Through other experiments Van Helmont came to believe that water was the main, if not the only ingredient in all substances. He also agreed with alchemy, the popular notion of the period, that cheaper metals could be changed into gold.

During Helmont's time more was known about the functioning of animals than was known about plants. Many believed the view of the ancient Greek philosopher Aristotle who stated that plants use food from the earth in order to grow. Van Helmont had a different idea that probably stemmed from his belief in alchemy. The following paper reports how Van Helmont tested his idea through experimentation.

BY EXPERIMENT THAT ALL VEGETABLE MATTER IS TOTALLY AND MATERIALLY OF WATER ALONE

JEAN BAPTISTE VAN HELMONT (1648)

That all vegetable matter immediately and materially arises from the element of water alone I learned from this experiment. I took an earthenware

pot, placed in it 200 lbs of earth dried in an oven, soaked this with water, and planted in it a willow shoot weighing 5 lbs. After five years had passed, the tree grown therefrom weighed 169 lbs and about 3 oz. But the earthenware pot was constantly wet only with rain or (when necessary) distilled water; and it was ample in size and imbedded in the ground; and, to prevent dust flying around from mixing with the earth, the rim of the pot was kept covered with an iron plate coated with tin and pierced with many holes. I did not compute the weight of the deciduous leaves of the four autumns. Finally, I again dried the earth of the pot, and it was found to be the same 200 lbs minus about 2 oz. Therefore, 164 lbs of wood, bark, and root had arisen from the water alone.

In Ortus Medicinae. Published by his son, Franz M. van Helmont. Amsterdam. 1648. Pp. 108-109: English Translation. Physick Refined, John Chandler. London. 1662.

Guide to Paper by Jean Baptiste van Helmont

1. *What hypothesis was van Helmont testing?*
2. *Briefly describe van Helmont's method.*
3. *Describe two measures that van Helmont took to increase the accuracy of his results.*
4. *Van Helmont concluded that "164 lbs of wood bark and root had arisen from the water alone." How would one explain this today?*
5. *What would have been the effect on the results if Van Helmont had computed "the weight of the deciduous leaves of the four autumns"?*

Joseph Priestley—An Early Chemist's Contribution to Biology

After five years as a minister to several congregations in England, Joseph Priestley (1733-1804) developed an interest in chemistry after attending a lecture by a prominent chemistry professor. Priestley's education was in philosophy and languages and completely ignored the sciences. He was a political activist who wrote many articles attacking British policy toward the American colonies, and he also supported the French Revolution. As a result of these unpopular views his house with its library and laboratory was burned and destroyed by an angry mob. Priestley

eventually came to the United States, where he lived in Pennsylvania for the last ten years of his life, enjoying the friendship of George Washington, John Adams, and Thomas Jefferson.

Priestley was naturally curious, and he worked quite unsystematically, testing and adding chemicals to almost everything which aroused his interest. As he lived next door to a brewery, Priestley decided to study the gas which was given off in the process of making beer. He discovered that by dissolving some of this gas in water, he produced a bubbly drink.

Priestley's curiosity about gases (he called them "different kinds of air") led him to perform many different experiments. In one investigation, he attempted to discover the nature of the air that remains after a candle burns. In the following paper one can see the first beginnings of modern chemistry and its influence upon the development of biology. Just as Van Helmont linked water with plant growth, Priestley's work relates gases and the atmosphere to living plants.

OBSERVATIONS ON DIFFERENT KINDS OF AIR

JOSEPH PRIESTLEY (1772)

I flatter myself that I have accidently hit upon a method of restoring air which has been injured by the burning of candles, and that I have discovered at least one of the restoratives which nature employs for this purpose. It is vegetation.

One might have imagined that, since common air is necessary to vegetable as well as to animal life, both plants and animals had affected it in the same manner, and I own that I had that expectation, when I first put a sprig of mint into a glass-jar, standing inverted in a vessel of water; but when it had continued growing there for some months, I found that the air would neither extinguish a candle, nor was it at all inconvenient to a mouse, which I put into it.

Finding that candles burn very well in air in which plants had grown a long time, and having had some reason to think that there was something attending vegetation, which restored air that had been injured by respiration, I thought it was possible that the same process might also restore the air that had been injured by the burning of candles.

Accordingly, on the 17th of August, 1771, I put a sprig of mint into a

quantity of air, in which a wax candle had burned out, and found that, on the 27th day of the same month, another candle burned perfectly well in it. This experiment I repeated, without the least variation in the event, not less than eight or ten times in the remainder of the summer. Several times I divided the quantity of air in which the candle had burned out, into two parts, and putting the plant into one of them, left the other in the same exposure, contained also, in a glass vessel immersed in water, but without any plant; and never failed to find, that a candle would burn in the former, but not in the latter.

This remarkable effect does not depend upon anything peculiar to mint, which was the plant that I always made use of till July 1772; for on the 16th day of that month, I found a quantity of this kind of air to be perfectly restored by sprigs of balm, which had grown in it from the 7th day of the same month.

In Philosophical Transactions of the Royal Society. London. Vol. 62. 1772. Pp. 166-170.

Guide to Paper by Joseph Priestley

1. *What hypothesis was Priestley testing?*
2. *Describe the controlled experiment that Priestley performed in order to test his hypothesis.*
3. *What were Priestley's observations to the above experiment?*
4. *What was Priestley's control in the experiment?*
5. *What conclusion did Priestley come to?*
6. *How would one explain Priestley's observations today?*
7. *Using modern terms, what would be the difference between what Priestley calls "injured air" and "restored air?"*

Jan Ingenhousz—The Beginning of Modern Ecology

A first important step toward the understanding of plant function was made by Van Helmont in 1648 when he demonstrated the role of water in the life of the plant. It took more than a century for Priestley to advance knowledge another step by revealing the role of gases in the process. With improvement in methods of scientific reporting, communication, and technology, it was possible for Priestley's work to be continued just a few years later by Jan Ingenhousz (1730-1799).

This Dutch physician to the Empress Maria Theresa of Austria eventually settled in London. During the summer of 1778 Ingenhousz performed more than 500 experiments with green plants. He introduced the concept of the balance between the animal and plant world which is the basis of modern ecology. As is the case with many investigators, Ingenhousz's work was not appreciated until many years later when the details of plant physiology were finally discovered.

EXPERIMENTS UPON VEGETABLES

JAN INGENHOUSZ (1779)

I was not long ago engaged in this enquiry before I saw a most important scene opened to my view: I observed, that plants not only have a faculty to correct bad air in six or ten days, by growing in it, as the experiments of Dr. Priestley indicate, but that they perform this important office in a complete manner in a few hours; that this wonderful operation is by no means owing to the vegetation of the plant, but to the influence of the light of the sun upon the plant.

This remarkable property of plants is indeed very great; for in a few hours, nay even sometimes in an hour and an half, they purify so much a body of air quite unfit for respiration, as to be equal in goodness to atmospheric air. They will even do it when they are inclosed in a glass vessel, without any water. One leaf of a vine, shut up in an ounce vial, full of air fouled by breathing so that a candle could not burn in it, restored this air to the goodness of common air in the space of an hour and a half. But plants enjoy this privilege only in the daytime, and when they grow in unshaded places.

Experiments tending to investigate to what degree plants may affect common air in the night, and by day time in shaded places:

Two handfuls of leaves of French beans were put in a jar of a gallon; it was kept inverted upon a dish, and some water poured upon it; next morning I found the air so much fouled that a candle could not burn in it.

After having taken out some of the air for trial, I placed the jar with the remaining air and leaves in the sun from nine till eleven o'clock, when I found the air so much mended, that a candle could burn in it.

After this I replaced it again in the sun till five in the afternoon, when I found the air so much mended as to be equal in goodness to common air.

Experiments showing that no part of plants improve ordinary air, or yield dephlogisticated air (oxygen), but the leaves and the green stalks:

I put some green stalks of a willow tree, the leaves being stripped off, in a gallon jar filled with pump-water; the jar was exposed, inverted, as ordinary, upon a wall in a warm sun-shine during four hours. They became most beautifully covered with an infinite number of round air bubbles. A great deal of dephlogisticated air was obtained.

Some branches of a mulberry tree, covered with grey bark, were put in a gallon jar full of pump-water, and exposed to the sun. A moderate quantity of air was obtained, which proved to be about the same quality with common air.

"Experiments on Vegetables." London. 1779.

Guide to Paper by Jan Ingenhousz

1. *In what ways did the work of Ingenhousz add to Priestley's work?*
2. *Why wouldn't a candle burn in air in a jar in which leaves were left over night?*
3. *What did Ingenhousz do to the above air so that a candle would burn in it?*
4. *What do Ingenhousz's observations about "One leaf of a vine, shut up in an ounce vial . . . ." tell us about the interrelations between plants and animals?*
5. *Ingenhousz mentions that a jar with water and green stalks when in sunlight becomes covered with "round air bubbles." What are these bubbles? Why were they formed?*
6. *What specific contribution was made by each of the investigators in this section to our understanding of photosynthesis?*

THE CONQUEST OF DISEASE

Edward Jenner—How a Country Doctor's Observation Led to the Elimination of Smallpox

At one time vast numbers of people in Europe died because of epidemics of different diseases. One such plague—the black death—is said to have taken the lives of 25 to 40 million persons in the Middle Ages from the years 1347 to 1349. Despite great advances in civilization during the next 400 years, populations were still in danger of being attacked by infectious diseases.

A disease known as smallpox was a major health problem when Edward Jenner (1749-1823) was born in England. This disease was also transported to the American colonies during the seventeenth century, severely attacking the American Indians as well as the new settlers.

The son of a clergyman, Jenner studied medicine and became a doctor although he had interests in geology, music, and poetry. One of his earliest jobs involved preparing and arranging zoological specimens collected on the voyage of Captain Cook to Australia in 1771. He settled in the English countryside where he noted variations in the ability of different groups of people to resist smallpox.

Dairymen and milkmaids never seemed to contract the disease. This observation led Jenner to formulate a hypothesis concerning a method of preventing it. He began his investigations in 1795, but it was not until 1796 that he was finally able to test this method. After the publication of Jenner's report describing his technique for preventing smallpox, it was rapidly adopted throughout Europe and the United States. In 1803, Jenner's work resulted in the annual average of deaths from smallpox falling from 2,000 to 600 in London alone.

Although Jenner developed a way of eliminating smallpox, he was unable to explain the basic reasons for the success of his

method. New knowledge had to be uncovered by other investigators in later years before explanations were possible.

AN INQUIRY INTO THE CAUSES AND EFFECTS OF A DISEASE DISCOVERED IN SOME OF THE WESTERN COUNTIES OF ENGLAND AND KNOWN BY THE NAME OF THE COW-POX

EDWARD JENNER (1798)

What renders the cow-pox virus so extremely singular is that the person who has been thus affected is forever after secure from the infection of the small-pox.

In support of so extraordinary a fact, I shall lay before my reader a great number of instances.

Joseph Merret lived as a servant with a farmer near this place in the year 1770, and occasionally assisted in milking his master's cows. The cows soon became affected with the cow-pox, and soon after several sores appeared on his hands. Swellings and stiffness followed, and he was too much indisposed for several days as to be incapable of pursuing his ordinary employment.

In April, 1795, Merret and his family were exposed to smallpox; so that a period of twenty-five years had elapsed from his having the cow-pox to this time. However, though material from smallpox sores was repeatedly inserted into his arm, I found it impossible to infect him with it. During the whole time that his family had the smallpox, one of whom had it very full, he remained in the house with them, but received no injury from exposure to the contagion.

It is necessary to observe that the utmost care was taken to ascertain, with the most scrupulous precision, that no one whose case is here reported had gone through the smallpox previous to those attempts to produce that disease.

Sarah Portlock, of this place, was infected with the cow-pox when a servant at a farmer's in the neighborhood, twenty-seven years ago.

In the year 1792, believing herself, from this circumstance, secure from the infection of the smallpox, she nursed one of her own children who had accidently caught the disease, but no indisposition ensued. During the time she remained in the infected room, variolous (smallpox) matter was inserted into both her arms, but without any further effect than in the preceding case.

The more accurately to observe the progress of the infection I selected a healthy boy, about eight years old, for the purpose of giving him cow-pox.

The matter was taken from a sore on the hand of a dairy maid, who was infected by her master's cows, and it was inserted on the 14th day of May, 1796, into the arm of the boy by means of two superficial incisions, each about an inch long.

On the seventh day he complained of uneasiness and on the ninth he became a little chilly, lost his appetite, and had a slight headache. During the whole of this day he was perceptibly indisposed, and spent the night with some degree of restlessness, but on the day following he was perfectly well.

In order to ascertain whether the boy, after feeling so slight an affection of the system from the cow-pox, was secure from the contagion of the smallpox, he was inoculated on the first of July following with variolous (smallpox) matter, immediately taken from a sore. Several slight punctures and incisions were made on both his arms, and the matter was carefully inserted, but no disease followed. The same appearances were observable on the arms as we commonly see when a patient has had variolous matter applied, after having either the cow-pox or smallpox. Several months afterwards he was again inoculated with variolous matter, but no effect was produced on his body.

To convince myself that the variolous matter made use of was in a perfect state I at the same time inoculated a patient with some of it who never had gone through the cow-pox, and it produced the smallpox in the usual regular manner.

Thus far have I proceeded in an inquiry founded, as it must appear, on the basis of experiment; in which, however, some assumptions have been made so that others may make more minute investigations. In the meantime I shall myself continue to prosecute this inquiry, encouraged by the hope of its becoming essentially beneficial to mankind.

"An Inquiry into Causes and Effects of Variolae Vaccine." S. Low. London. 1798.

Guide to Paper by Edward Jenner

1. *What hypothesis was Jenner testing?*
2. *How was his hypothesis supported by the cases he reported in the paper?*
3. *What two indications are there that Jenner was a cautious and careful investigator?*
4. *What experimental control was described by Jenner?*
5. *What presently used method of disease prevention is Jenner actually describing?*

Louis Pasteur—From Preventing Diseases of Wine to Preventing Diseases of Man

Each glass of milk that you drink serves as a continuing reminder of the work of the famous French microbiologist, Louis Pasteur (1822-1895). He was born in a small town, the son of a former soldier in Napoleon's army who became a tanner after his military service. Young Pasteur was interested in the natural sciences and decided to follow a teaching career.

He became a professor of chemistry and investigated fermentation, which is a process involved in the souring of milk and in the production of wine. Businessmen supplying milk and wine frequently found their products to be "diseased" due to problems in controlling fermentation. They turned to Pasteur for assistance. He examined samples of fermenting solutions under the microscope and discovered that the process of fermentation was a result of the activity of several different kinds of microorganisms. Pasteur demonstrated a method of controlling fermentation through the use of heat, and this process of "pasteurization" has eliminated the risk of disease from microorganisms present in milk.

In 1865, Pasteur was called to the south of France to deal with the disease of silkworms which was ruining the French silk industry. After three years of investigation, Pasteur isolated the microorganisms producing the disease and found a method of controlling them. This discovery not only saved the French silk industry but also those of all silk-producing countries.

After returning to Paris, Pasteur turned his attention to the disease of anthrax, which affected sheep, and cholera, which affected chickens. In each case he isolated the germ causing the disease and developed methods of producing immunity to both. He called his method vaccination in remembrance of the work of Edward Jenner who used cowpox material to prevent smallpox.

Pasteur's work became known throughout the world. His germ theory of disease revolutionized medical practices. Yet he maintained a simple style of life, overcame the handicap of paralysis at the age of 46, and continued his work until his death. Contributions from people in France and in many other countries established the Pasteur Institute in 1888, which, to this day,

conducts investigations leading to the conquest of disease. The accompanying paper describes how Pasteur attempted to prevent the development of rabies in a child bitten by a dog.

PREVENTION OF RABIES

LOUIS PASTEUR (1885)

Progress in the study of rabies was made when I announced a preventive method. But the progress was scientific rather than practical. Accidents were liable to occur in its application. Of twenty dogs treated, I could not make more than fifteen or sixteen resistant to rabies.

The method did not lend itself easily to the immediate treatment made necessary by the accidental and unforeseen way in which bites are inflicted by rabid animals.

It was necessary, therefore, to discover, if possible, a more rapid method, and yet one capable of giving perfect security to dogs. Otherwise who would have the courage, before this progress had been achieved, to make any experiment on man?

After making almost innumerable experiments, I have discovered a preventive method which is practical and prompt. The results in dogs are sufficiently numerous, certain, and successful to warrant my having confidence in its general applicability to all animals, and even to man itself.

A dog may be made immune to rabies in a relatively short time in the following way:

Every day pieces of fresh diseased spinal cord from a rabbit which has died of rabies are dried in a series of flasks. Every day a dog is inoculated under the skin with a small piece of one of the spinal cords. The first inoculation is with a spinal cord that has been dried long enough to make it certain that the cord was not able to produce rabies. (This period of time had been determined by previous experiments.) On the following days the same operation is performed with cords dried for shorter periods of time until at last a very diseased cord, which has only been dried for two days, is used.

The dog has now been made immune to rabies. It may be inoculated with the virus of rabies under the skin, or even on the surface of the brain, without any subsequent development of rabies.

Never having once failed when using this method, I made fifty dogs, of all ages and of every race, immune to rabies, when three individuals from Alsace unexpectedly presented themselves at my laboratory, on Monday the 6th of last July.

Joseph Meister, aged 9 years, was bitten on July 4th, at eight o'clock in the morning, by the grocer's dog. This child had been knocked over by the dog and suffered numerous bites, on the hands, leg, and thighs, some of them so deep as to make walking difficult.

At the examination of the dog, after its being killed by its master, its stomach was found full of hay, straw, and scraps of wood. The dog was certainly rabid. Joseph Meister had been pulled out from under him covered with foam and blood.

Since the death of this child appeared to be inevitable, I decided, not without fear, to try upon Joseph Meister the method which I had found constantly successful with dogs.

Consequently, on July 6th, at 8 o'clock in the evening, sixty hours after the bites on July 4th, young Meister was inoculated under a fold of skin with half a syringeful of the spinal cord of a rabbit which had died of rabies on June 21st. It had been preserved since then, that is to say, fifteen days, in a flask of dry air.

On the following days fresh inoculations were made. I thus made thirteen inoculations, and prolonged the treatment to ten days. I shall say later on that a smaller number of inoculations would have been sufficient. But it will be understood how, in the first attempt, I would act with a very special circumspection.

The days following, new inoculations were made, according to the conditions I give in the following table:

July	Time	Cord Taken	Dried for
7th	9 am	June 23rd	14 days
7th	6 pm	June 25th	12 days
8th	9 am	June 27th	11 days
8th	6 pm	June 29th	9 days
9th	11 am	July 1st	8 days
10th	11 am	July 3rd	7 days
11th	11 am	July 5th	6 days
12th	11 am	July 7th	5 days
13th	11 am	July 9th	4 days
14th	11 am	July 11th	3 days
15th	11 am	July 13th	2 days
16th	11 am	July 15th	1 day

In order to determine how diseased the spinal cords were, two healthy rabbits were inoculated with the various spinal cords.

Observation of these rabbits enabled us to see that the spinal cords of July 7th, 8th, 9th, 10th, were not diseased for they did not give the rabbits rabies. The spinal cords of July 11th, 12th, 14th, 15th, 16th, were all diseased and the rabies material was present in larger and larger proportion. Rabies appeared after seven days in the rabbits of July 15th and 16th, after eight

days in those of the 12th and 14th and after fifteen days in those of July 11th.

On the last days, therefore, I had inoculated Joseph Meister with the most powerful material of rabies. When the condition of immunity has been attained, the most powerful rabies material can be inoculated, in considerable quantity, without ill effects. Joseph Meister, therefore, has escaped the rabies which would have been caused by the bites he received. He also did not develop rabies from the material with which I have inoculated him, this rabies being even more powerful than the ordinary rabies transmitted by dogs.

In Recent Essays on Bacteria in Relation to Disease. W. W. Cheyne, Translator. New Sydenham Society. London. 1886.

Guide to Paper by Louis Pasteur

1. *Why was Pasteur not satisfied with his first attempt to prevent rabies?*
2. *What properties did Pasteur think an effective method of preventing rabies should have?*
3. *Describe the method developed by Pasteur.*
4. *Why did Pasteur dry the pieces of spinal cord for various periods of time?*
5. *Why did Pasteur inject the diseased spinal cord material into two healthy rabbits?*
6. *Suppose someone that you know is seriously bitten by a dog. What would you recommend that this person do?*

Joseph Lister—Reducing Deaths on the Operating Table

In reviewing the careers of famous scientists, one notices that many investigators were raised in ordinary homes where there was no emphasis on science. Very often it was only an extreme natural curiosity that drew them into the world of science. Joseph Lister (1827-1912) did not fit into this pattern. His father was an English scientist who greatly improved the compound microscope by increasing the sharpness of its image.

Joseph Lister completed his studies at the University of London where he received degrees as a doctor and as a surgeon. In the 1860s, there was very little chance of a patient surviving surgery as infections were contracted during the operation. It is estimated

that four out of every five operations ended with the death of the patient. Operating rooms of that time usually had sawdust on the floor to absorb blood, and the surgeons often wore gowns covered with dried blood and pus.

Lister tried to change these conditions. In the course of his research, he read of Pasteur's work describing microorganisms which are found in the air. Lister then developed the hypothesis that these microorganisms from the air and from the surgeon's hands and instruments caused the infections which led to the death of so many patients. He therefore attempted to find a technique of destroying these organisms.

Doctors and nurses were slow to adopt his methods, but eventually his success in reducing the death rate in surgery to 15 percent in only three years removed their doubts. In his later life, Lister was honored by the British government which established the Lister Institute of Preventive Medicine as a tribute to his work.

ON A NEW METHOD OF TREATING COMPOUND FRACTURE

JOSEPH LISTER (1867)

The frequency of disastrous consequences in compound fracture, contrasted with the complete immunity from danger to life or limb in a simple fracture is one of the most striking as well as melancholy facts in surgical practice.

If we inquire how it is that an external wound in contact with a fracture leads to such grave results, we cannot but conclude that it is through the effect of the atmosphere that decomposition of the blood occurs.

We know that blood kept exposed to the air at the temperature of the body, in a vessel of glass soon decomposes. There is no reason to suppose that the living tissues surrounding a mass of externally flowing blood could preserve the blood from being affected in a similar manner by the atmosphere. On the contrary, it may be ascertained as a matter of observation, that, in a compound fracture, twenty-four hours after the accident the colored serum which oozes from the wound is already distinctly tainted with the odor of decomposition.

Turning now to the question how the atmosphere produces decomposition of organic substances, we find that a flood of light has been thrown upon this most important subject by the philosophic researches of Pasteur, who has

demonstrated by thoroughly convincing evidence that it is not to its oxygen or to any of its gaseous constituents that the air owes this property, but to minute particles suspended in it, which are the germs of various low forms of life, long since revealed by the microscope, but now shown by Pasteur to be its essential cause, breaking down the complex organic compounds into substances of simpler chemical composition.

Applying these principles to the treatment of compound fracture, bearing in mind that it is from the vitality of the atmospheric particles that all the mischief arises, it appears that all that is necessary is to dress the wound with some material capable of killing these septic germs, provided that any substance can be found reliable for this purpose, yet not too strong.

The material which I have employed is carbolic or phenic acid, a volatile organic compound, which appears to exercise a peculiarly destructive influence upon low forms of life, and hence is the most powerful antiseptic with which we are at present acquainted.

The first class of cases to which I applied it was that of compound fractures, in which the effects of decomposition in the injured part were especially striking. The results have been such as to establish conclusively the great principle that all local inflammatory mischief which follow severe injuries are due to the irritating and poisonous influence of decomposing blood. For these evils are entirely avoided by the antiseptic treatment, so that limbs which would otherwise be unhesitatingly condemned to amputation may be retained, with confidence of the best results.

In conducting the treatment, the first object must be the destruction of any septic germs which may have been introduced into the wounds, either at the moment of the accident or during the time which has since elapsed. This is done by introducing the acid of full strength into all accessible recesses of the wound by means of a piece of rag held in dressing forceps and dipped into the liquid.

"On the Antiseptic Principle of the Practice of Surgery." British Medical Journal *II:246-248; September 21, 1867.*

Guide to Paper by Joseph Lister

1. *What was Lister's hypothesis?*
2. *How did the work of Pasteur help Lister?*
3. *Why did Lister recommend the use of substances such as carbolic or phenic acid in the treatment of wounds?*
4. *How were surgical procedures changed as a result of Lister's work?*
5. *What present-day methods of treating wounds in the home are related to Lister's work?*
6. *What newer approach in today's surgery is used to supplement Lister's method of preventing infections?*

Robert Koch—The Father of Bacteriology

As so often happens in science, three scientists in three different countries were independently investigating similar problems at the same time. While Louis Pasteur was in his laboratory in France, and Joseph Lister was working in a hospital in Great Britain, the son of a German miner, Robert Koch (1843-1910), was busy with his duties as a country doctor. A serious outbreak of the disease anthrax among cattle presented a new problem to be solved. He, therefore, turned his attention away from the treating of patients to the study of diseases and their causes.

In 1876, Koch discovered that anthrax was caused by rod-shaped bacteria, and seven years later he developed a method of preventive inoculation against it. Koch developed many precise techniques for the investigation of bacteria. He was the first to isolate pure strains of bacteria and to grow them on a culture medium solidified with gelatin. He also perfected methods of staining bacteria with dyes for greater visibility and was able to take photographs of them through his microscope.

In the course of his work, Koch developed an experimental method to prove that a specific bacterium causes a specific disease. This method, referred to as Koch's Postulates, is standard procedure today for all scientists who study disease-producing microorganisms. He helped to establish a school of bacteriology in Berlin which attracted young students throughout the world. Later, many of them were able to continue and extend his work. In 1905, he was awarded the Nobel Prize for Medicine for his contributions to bacteriology and his investigation of the specific disease which is described in the following paper.

THE AETIOLOGY OF TUBERCULOSIS

ROBERT KOCH (1882)

There have been repeated attempts to understand the nature of tuberculosis, but thus far without success. The so frequently successful staining methods for the demonstration of pathogenic micro-organisms have failed in regard to this disease, and, to date, the experiments designed to isolate and cultivate a tubercle virus cannot be considered successful.

Cohnheim, in the recently published and newest edition of his lectures on general pathology had to designate "the direct demonstration of the tuberculosis virus as a still unsolved problem."

In my studies on tuberculosis I first used the known methods without discovering the nature of the disease. But by reason of several incidental observations I was prompted to abandon these methods and to follow other paths which finally led to positive results.

The aim of the study had to be directed toward the demonstration of some kind of parasitic forms, which are foreign to the body and which might possibly be interpreted as the cause of the disease. This demonstration became successful, indeed, by means of a certain staining process, which disclosed characteristic and heretofore unknown bacteria in all organs affected by tuberculosis.

In several respects the bacteria made visible by this process exhibit a characteristic behavior. They are rod-shaped, and they belong to the group of bacilli. They are very thin and one-fourth to one-half as long as the diameter of a red blood corpuscle, although they may sometimes reach a greater length, up to the full diameter of the erythrocyte.

In addition to cases of spontaneous tuberculosis, I studied a large number of animals which had been injected with the most varied tuberculosis materials. The number of animals so infected amounted to 172 guinea pigs, 32 rabbits and five cats. In the majority of these cases the demonstration of bacilli had to be limited to the lungs. Here bacilli never failed to be found.

On the basis of my numerous observations I consider it established that, in all tuberculosis cases of man and animals, there occur constantly these bacilli which I have designated tubercle bacilli and which are distinguishable from all other micro-organisms by characteristic properties. However, from the mere coincidental relation of tuberculosis cases and bacilli it may not be concluded that these two phenomena have a cause and effect relationship. There is considerable likelihood that this assumption is true as the bacilli occur in organs affected by tuberculosis and they disappear when the disease comes to a standstill.

To prove that tuberculosis is a parasitic disease, that it is caused by the invasion of bacilli and that it is conditioned primarily by the growth and multiplication of the bacilli, it was necessary to isolate the bacilli from the body; to grow them in pure culture until they were freed from any disease product of the animal organism which might adhere to them; and by administering the isolated bacilli to healthy animals, to reproduce the same diseased condition which is obtained by injection with spontaneously developed tuberculosis material.

Of 8 guinea pigs, 6 were inoculated with bacillary culture material, derived from the tuberculosis lung of an ape, and cultivated in eight cultures for

ninety-five days. Two animals remained uninoculated as controls. At autopsy the 6 inoculated animals were found with far advanced tuberculosis, while the two non-inoculated ones were healthy when they were killed, after thirty-two days.

"On the Investigation of Pathogenic Organisms." In Recent Essays on Bacteria in Relation to Disease. Victor Horseley, Translator. New Sydenham Society, London, 1886. Also in "Aetiology of Tuberculosis." American Review of Tuberculosis. National Tuberculosis and Respiratory Disease Association, New York, 1932.

Guide to Paper by Robert Koch

1. *What problem did Koch attempt to solve?*
2. *What was his hypothesis?*
3. *What experimental technique did Koch have to develop before he could test his hypothesis?*
4. *Describe the bacteria which Koch observed.*
5. *In what two different ways did Koch test his hypothesis?*
6. *What conclusions did Koch reach concerning the cause of tuberculosis?*

Walter Reed—How a Doctor Helped Build the Panama Canal

In 1898 the United States went to war with Spain. One reason, among many others, for this war, was the American desire to make Spain give up its colony in Cuba. United States forces were sent to that island in order to remove the Spanish troops. As the war continued, casualty reports indicated that as many soldiers died from the disease yellow fever as died from battles. After the war, in 1900, the United States government sent a team of four army surgeons, led by Dr. Walter Reed (1851-1902), to investigate the cause of this disease, so it could be eliminated from Cuba.

Walter Reed was born in Virginia. After completing medical school, he entered the United States Army Medical Corps. He rose to the position of Professor of Bacteriology of the newly established Army Medical School.

A problem that Reed and his colleagues faced in studying yellow fever was that this disease did not affect animals. Therefore it was necessary to experiment on humans. It was during the course of some of these experiments that two doctors working with Reed contracted yellow fever and died. However, Reed was able to discover how the disease was transmitted, and by the year 1901 there was not a single case of yellow fever in Havana.

Armed with this information, public health officers were then able to rid the world of yellow fever. As this disease was one of the main obstacles to building the Panama Canal, William Gorgas was appointed by President Theodore Roosevelt to make the Canal Zone yellow-fever free. That this task was accomplished in only two years is in large part due to the pioneering work of Dr. Walter Reed.

THE PROPAGATION OF YELLOW FEVER; OBSERVATIONS BASED ON RECENT RESEARCHES

WALTER REED (1901)

In the ordinary course of army administration, I found myself brought in contact with yellow fever during the summer of 1900, under such circumstances as permitted me to give my entire time to the study of its etiology and propagation . . .

As the good health of those who were occupying the "Infected Clothing Building" pointed strongly to the harmlessness of fomites, the next experiment at this station was undertaken for the purpose of demonstrating that the essential factor in the infection of a building with yellow fever is the presence therein of mosquitoes that have bitten cases of yellow fever.

Accordingly at 11:55 A.M., December 21, 1900, fifteen mosquitoes were freed in the larger room of the "Infected Mosquito Building" which, as I have said, was divided into two compartments by a wire-screen partition. The interval that had elapsed since the contamination of these insects was as follows: one, twenty-four days; three, twelve days; four, eight days; and seven, five days. The only articles of furniture in this building consisted of three beds, one being placed in the mosquito room and two beyond the wire screen, these latter intended to be occupied by two "control" non-immunes. The articles of bedding as well as the bedsteads had been carefully disinfected with steam. At noon on the same day, five minutes after the mosquitoes had been placed therein, a plucky Ohio boy, clad only in his night shirt, and fresh

from a bath, entered the room containing the mosquitoes, where he lay down for a period of thirty minutes. On the opposite of the screen were the two "controls" and one other non-immune. Within two minutes from the boy's entrance he was being bitten about the face and hands by the insects that had promptly settled down upon him. Seven in all bit him at this visit. At 4:30 P.M., the same day, he again entered and remained twenty minutes, during which time five others bit him. The following day at 4:30 P.M., he again entered and remained fifteen minutes, during which time three insects bit him, making the number fifteen that had fed at these three visits. The building was then closed, except that the two non-immune "controls" continued to occupy the beds on the non-infected side of the screen. On Christmas morning at 11 A.M., this brave lad was stricken with yellow fever, and had a sharp attack, which he bore without a murmur. The period of incubation in this case was three days and twenty-three hours, counting from his first visit, or two days and seventeen and a half hours, if reckoned from his last visit. The two "controls" who had slept each night in this house, only protected by the wire screen, but breathing the common atmosphere of the building, had remained in good health. They continued to so remain, although required to sleep here for thirteen additional nights.

etiology - theory of the cause of a disease
fomites - substances which contain germs and spread disease

"Propagation of Yellow Fever." Medical Records. *Vol. 60, No. 6, August 10, 1901.*

Guide to Paper by Walter Reed

1. *What inference did Reed make from the observation that persons who occupied the "Infected Clothing Building" remained in good health?*
2. *What hypothesis was Reed testing?*
3. *What does Reed mean by "contaminated insects?"*
4. *Describe the experiment carried on in the "Infected Mosquito Building."*
5. *Describe the ways in which the above experiment was controlled.*
6. *Did the results of the above experiment confirm the hypothesis of Reed? Explain your answer.*

Paul Ehrlich—Chemical Bullets to Fight Disease

As progress was being made toward the elimination of many infectious diseases in the last half of the nineteenth century,

investigators began to realize that there were some diseases to which people could not be made immune. The German scientist Paul Ehrlich (1854-1915) attempted to solve this problem.

His academic training included medicine, chemical research, and work in the field of bacteriology. He became interested in the effects of chemicals on cells as a result of his research concerning methods of staining cells with different kinds of chemical dyes. Although he had to suspend his studies for two years because of tuberculosis, Ehrlich was able to recover and continue experimentation at Robert Koch's Institute for Infectious Diseases in Berlin.

At the Institute, Ehrlich engaged in the study of immunology and was attracted to studying the ways in which antibodies, chemicals produced by the body, could destroy microorganisms. He thought of these antibodies as "magic bullets" which seek out and kill germs that enter the body. Recalling his earlier work on dyes, Ehrlich considered the possibility of using chemicals as "magic bullets" which could fight disease.

He began to treat diseases by the use of chemicals, thereby creating the new field of chemotherapy. In 1910, Ehrlich prepared a compound of the element arsenic called salvarsan which proved effective in curing syphilis. As it was the 606th compound that he had tried before achieving success, this substance is known as "606." A pioneer in the field of immunology, for which he received the Nobel Prize in 1908, Ehrlich developed the principles of chemotherapy upon which the later use of the life-saving sulfa drugs and antibiotics is based.

MODERN CHEMOTHERAPY

PAUL EHRLICH (1908)

I now come to an extremely important point concerning the cause of the action of arsanil which has until now been clothed in darkness. Arsanil brings about a wonderfully rapid and surprising disappearance of the parasite in experimental as well as in natural infections with trypanosomes (protozoans), especially in the case of sleeping sickness.

It seemed very probable that arsanil brought about directly the death of the parasites. However, while a number of other trypanosome active agents

kill completely the trypanosomes in the test tube, such is not the case with arsanil.

It may be either that the body modifies the compound in some way, or that it induces in the body the production of some other substance which kills trypanosomes.

About one-and-a-half to two years ago I was able to shed a little light on the mode of action of arsanil, and the observations which first showed me the way were really quite ordinary. In my first studies I stated that arsanil displayed no reproducible effect in mice. While in general the tolerated dose for our mice was 1/300 solution, there were mice that were able to tolerate double this concentration without any symptoms, while there were others which became sick from quite small doses, for example, 1/400, and could even die.

It seemed to me probable that this was not due to uncontrolled variables, but was a characteristic of the individual mice, since I was able to show that mice which had once survived a dose of 1/150, could tolerate a second injection of this same concentration.

It seemed probable that in the organism the arsanil was being converted into another substance which was toxic, but which at the same time exerted a strong effect on the parasites.

In Milestones in Microbiology, T. D. Brock, Editor and Translator. Prentice-Hall, Inc., Englewood Cliffs, New Jersey. 1961. From Beitrage zur Experimentellen Pathologie und Chemotherapie. 1909. Akademische Verlagsgesellschaft. Leipzig. Pp. 167-202.

Guide to Paper by Paul Ehrlich

1. *Why is the chemical arsanil effective in combating sleeping sickness?*
2. *Ehrlich believed that arsanil did not kill the protozoan parasites directly. Why?*
3. *What two possible explanations did Ehrlich consider for the way in which arsanil acts?*
4. *Why did Ehrlich believe the ability of a mouse to withstand the effects of arsanil was due to a characteristic of the individual mouse itself and not to the amount of arsanil used?*
5. *What hypothesis as to how arsanil acts had still to be tested by Ehrlich?*

Alexander Fleming—Chance and the Prepared Mind

Every bacteriologist has probably had the experience of having his culture plates exposed to some kind of contamination. Usually

these contaminated plates are discarded and new cultures are grown. In September 1928 Alexander Fleming decided to keep some of his contaminated culture plates and to study them further.

Alexander Fleming (1881-1955) was born in Darvel, Scotland. He excelled in his studies at the University of London Medical School, and after his graduation he entered the field of medical research. He became interested in antiseptics and methods of preventing bacterial action.

After completing military service in World War I, Fleming returned to his study of problems of infection. In 1922, he discovered lysozyme—a powerful antibacterial agent found in human tissues and secretions, such as tears.

Fleming continued his laboratory work, and in 1928 he made his most important discovery. It was Fleming who, by his discovery of penicillin, started the age of antibiotics. The use of these chemicals is so widespread today that all of us probably have been treated with them to combat disease at one time or another. In recognition for this great achievement Fleming was knighted by the British Government and awarded the Nobel Prize for Medicine in 1945.

ON THE ANTIBACTERIAL ACTION OF CULTURES OF A PENICILLIUM

ALEXANDER FLEMING (1929)

While working with staphylococcus a number of culture plates were set aside on the laboratory bench and examined from time to time. In the examinations these plates were necessarily exposed to the air and they became contaminated with various micro-organisms. It was noticed that around a large colony of a contaminating mould from the air, the staphylococcus colonies became transparent and were obviously being destroyed.

Subcultures of this mould were made and experiments conducted with a view to ascertaining something of the properties of the bacteria-destroying abilities of the substance which had evidently been formed in the mould culture and which had diffused into the surrounding medium. It was found that the broth in which the mould had been grown at room temperature for

one or two weeks had acquired great power to destroy many of the more common pathogenic bacteria.

For convenience, and to avoid using the phrase "mould broth substance" the name penicillin will be used for this antibacterial substance.

The simplest method of examining for this power is to make a slit in an agar plate and fill this in with a mixture of equal parts of agar and the broth in which the mould has grown. When this has solidified, cultures of various microbes can be streaked at right angles from the slit to the edge of the plate. The antibacterial substance produced by the mould diffuses very rapidly in the agar, so that in the few hours before the microbes show visible growth it has spread out for a centimeter or more in sufficient concentration to inhibit growth of a sensitive microbe. On further incubation it will be seen that the nearest portion of the culture for about one centimeter becomes transparent. On examination of this portion of the culture, it is found that practically all the microbes are dissolved, indicating that the antibacterial substance has continued to diffuse into the agar in sufficient concentration to dissolve the bacteria. This simple method therefore demonstrates the antibacterial properties of the mould culture, and also by the extent of the area of inhibition gives some measure of the sensitiveness of the particular microbe tested.

The toxicity to animals of penicillin appears to be very low. Twenty c.c. injected intravenously into a rabbit were not more toxic than the same quantity of broth. Half a c.c. injected intraperitoneally into a mouse weighing about 20 gm. induced no toxic symptoms. Constant application to large infected surfaces in man was not accompanied by any toxic symptoms, while application to the membrane of the eye every hour for a day had no irritant effect.

British Journal of Experimental Pathology
10:226-236; 1929.

Guide to Paper by Alexander Fleming

1. *The element of chance has played a part in scientific discovery more than once. How is this illustrated by Fleming's work?*
2. *Pasteur once remarked that "Chance favors the prepared mind." What do you think he meant?*
3. *What hypothesis did Fleming develop as a result of his observation?*
4. *What did Fleming do in order to test this hypothesis?*
5. *What were his observations?*
6. *Why do you think Fleming attempted to determine whether penicillin was toxic to animals?*

Jonas E. Salk—Preventing Polio with Killed Virus

The beginnings of the modern conquest of disease can be traced back to Jenner's work in 1798. Since that time many diseases that affected large numbers of people have been brought under control. Recently—probably within the memories of your parents or older brothers and sisters—a disease that attacked mainly children in epidemic proportions was eliminated.

This disease, poliomyelitis (polio) caused paralysis of arms and legs and of the muscles involved in breathing. For years polio was ignored by scientists who were occupied in attempting to prevent such fatal diseases as diptheria, typhoid, and tuberculosis.

However, in 1916, national attention was focused on polio when an outbreak of the disease resulted in 27,000 cases and 6,000 deaths in the United States. In certain areas people panicked and tried to leave their cities for smaller, unaffected towns. In some cases, armed citizens turned them away in fear that the strangers would infect the local children.

Five years later, national attention was again directed to polio when a promising young politician, Franklin D. Roosevelt was stricken with this disease. Despite the handicap of being confined to a wheelchair and having to wear steel braces on his legs, Roosevelt was elected Governor of New York in 1928 and President of the United States in 1932.

With the aid and encouragement of President Roosevelt, a national campaign was begun to find a way of preventing the dreaded disease. A National Foundation was formed to raise funds for the needed research. Some of this money was used to support the work of Jonas E. Salk (1914-).

Salk entered The City College of New York at the age of 15, after graduating from a special high school for bright students. As his parents were not financially well off, he won a scholarship to medical school. Although he completed his studies as an MD, Salk did not want to become a practicing physician.

He started by studying virus diseases, and eventually was appointed virologist at the University of Pittsburgh Medical School. There he classified the different strains of polio virus and developed techniques to grow polio virus in the kidney tissue of

monkeys. Aided by a grant from the National Foundation, Salk discovered a method of making humans immune to poliomyelitis by using killed polio virus.

In the paper which follows, Salk reports on his work while it was still in progress. One year later, a massive field testing program was undertaken.

RECENT STUDIES ON IMMUNIZATION AGAINST POLIOMYELITIS

JONAS E. SALK (1953)

Some six months ago we reported the results of experiments in progress on immunization of human subjects against poliomyelitis. In that report it was shown that it was possible to induce the formation of poliomyelitis antibody using tissue culture fluids treated with formalin and administered intramuscularly.

The question can justifiably be asked, "Why can't we try these methods of conferring antibody actively, by the use of an experimental vaccine, even in the present imperfect stage of development, to see if paralysis can be prevented?" The reply would be that if, with material that has been produced and processed on a large scale basis, antibody can be induced consistently and in as close to 100% of persons as possible, and if this effect can be expected to persist over a period of one season, at least, then there are no good reasons for not doing so, as long as the public at large, as well as our colleagues, understand that this is precisely what we are doing. We do not want to allow any distortion of the fact that we are still actively in the stage of clinical investigation; and that this work must be continued, but it must be continued gradually and cautiously.

If and when a study is made to determine the effectiveness of any vaccination procedure, there are several approaches from which to choose. One is that of a controlled study involving the injection of alternate children with either vaccine or a control preparation consisting of either another biologic preparation such as a vaccine for another disease, or of physiologic salt solution. This method has the incomparable advantage that it is "strictly scientific" in the orthodox sense and would allow a comparison of two strictly comparable groups without any possibility of bias in diagnosis in the follow-up period. Such a study could be conducted with the participation of children of elementary school age in widely scattered communities.

Another type of controlled study might involve the use of the poliomyelitis vaccine under test in children of a narrowly restricted age group, preferably the one of highest incidence, and that in this way information be accumulated relative to efficacy in preventing the paralytic disease; this would be done through an analysis of age specific attack rates within each vaccinated community, as well as comparisons between vaccinated and unvaccinated siblings. For simplicity of administration and greatest efficiency in deriving the most information, such observations could best be made in children in the first grade in schools in many parts of the country. In a study of this type the controls would consist of: (1) younger and older siblings of the vaccinated children; (2) younger and older children in the same school or community; (3) children of the same age in other communities; (4) past experience in children of the vaccinated age group in the community selected for study. If no protective effect is observed the testing of vaccination in this way can be stopped, or it can be continued with modifications until success is achieved.

Through either approach an answer is assured but "when" can not be predicted. Questions that may be raised concerning the persistence of effect beyond one season, and the need for booster doses at long intervals must remain as secondary until the primary questions have been answered concerning the protective value for one season.

It is necessary to give due attention to information that is given to the public that provides them with the basis for the attitude that will prevail. If a well controlled plan is to be evolved we must emphasize that a vaccine for general use is not yet here. Problems relative to mass production are being resolved rapidly in several laboratories, and the clear indications at present are that this may be one of the simplest biologic preparations to make. The fact that, at the moment, monkey-kidney tissue is required is not a problem, as is imagined by many, because relatively large amounts of potential vaccine can be derived from the tissue of a single animal.

The simple fact is: That an experimental method for inducing measurable amounts of antibody for the three known poliomyelitis viruses, employing a killed-virus vaccine is available, and it now becomes possible to determine whether—and to what extent—the incidence of naturally occurring paralysis may be influenced.

All that should be inferred now is that studies are progressing satisfactorily; there have been no setbacks nor anything but revelations that shed more light on the course ahead. We must continue to regard the experimental developments to date as providing immunologic markers along the way that tell us whether we are on the right road. That there is more to do now than before indicates that we have not stumbled down a by-way but have selected

a road, with many lanes, that seems long indeed. Our problem is to select not only the fast lane but the one that is safest and most certain.

formalin—also known as formaldehyde, an organic compound capable of killing certain viruses

Pediatrics 12:471-482; November 1955.

Guide to Paper by Jonas E. Salk

1. *What evidence is there to indicate that Salk is a cautious investigator?*
2. *According to Salk, what characteristics must a vaccine have before being given to humans as a means of preventing disease?*
3. *What methods of testing the effectiveness of his vaccine does Salk suggest?*
4. *Why is Salk concerned about information given to the public about his vaccine?*
5. *What is the role of monkey-kidney tissue in the preparation of poliomyelitis vaccine?*
6. *Why did Salk use killed virus in his vaccine?*

Thomas Francis—Testing the Salk Vaccine

When Jonas Salk was studying medicine at New York University, he studied bacteriology with Thomas Francis (1900-1969). Francis believed it possible that killed-virus vaccines could be developed and could be as effective as killed-bacteria vaccines. It was this view which helped shape Jonas Salk's career.

Thomas Francis was born in Indiana, the son of a Methodist minister, who was also a part-time steel worker. During the influenza epidemic of 1917, young Francis did volunteer work in the hospitals. He quickly decided upon a medical career, with an emphasis on the study of influenza.

After being graduated from Yale University School of Medicine, he later became director of influenza research at the Rockefeller Institute. In 1933, when influenza was identified as a viral disease, Francis shifted his research into investigating strains of virus.

At New York University, where he first meet Salk, Francis attempted to produce an influenza vaccine using dead influenza

virus. He continued this work at the University of Michigan. Shortly after the beginning of World War II, Jonas Salk joined him at Michigan as a research assistant.

The United States Army asked Francis to develop an influenza vaccine, as it did not wish to suffer the 44,000 soldier deaths from influenza that had occurred in World War I. Together, Francis and Salk developed a killed-virus vaccine which proved effective—although booster shots were needed.

Francis continued his work at the University of Michigan, and Salk left for the University of Pittsburgh. When Salk's polio vaccine was ready for field testing, the National Foundation turned to Thomas Francis. He agreed to conduct the study, but only after assuring the most rigorous of testing conditions. On April 12, 1955 (the 10th anniversary of the death of President Franklin D. Roosevelt) Thomas Francis announced the results of these field tests.

EVALUATION OF THE 1954 POLIOMYELITIS VACCINE (SALK) FIELD TRIAL

THOMAS FRANCIS, JR. (1955)

The evaluation program embraced two distinct plans. The plan originally announced by the National Foundation for Infantile Paralysis was to vaccinate all children in the second grade of school whose parents requested it, while all the children of the first and third grades were to be kept under observation as controls for the vaccinated children. The ease of carrying out vaccination under such conditions recommends the procedure, but it presents possible disadvantages in the evaluation by the ready opportunity for the introduction of bias at any stage in the diagnosis and investigation of cases.

The vaccinated persons are those who specifically volunteer; the controls comprise both those who might volunteer and those who would not. The age groups do not correspond, although the first and third grades bridge the second grade in this respect. It can readily be determined whether a child was vaccinated or not, and that knowledge could influence subsequent consideration.

In order to achieve strict impartiality in the study of patients in test and control groups, the second plan of study was introduced. It comprised all children of the first, second, and third grades whose parents requested their participation, with a clear understanding that half would receive vaccine and

the other half a placebo, while knowledge of the inoculum was available only by a code held at the evaluation center.

Those who received the placebo were the controls and have been shown in all respects to be counterparts of the vaccinated children. Those who did not participate were not considered additional controls. The uninoculated persons were, however, to be followed in the same manner as the participants; it was also to be true of uninoculated persons in the second grade of observed areas. This was adopted to gain uniformity of investigative procedure without consideration of vaccination status and to avoid selective problems at the local level. But it should be emphasized that those not included as participants are not an integral part of the evaluation. they are a different population.

Evidence that they are different was clearly supplied in a survey of the socioeconomic status and the reaction to health problems among families of the placebo study areas. Numerous studies have indicated differences in antibody development and susceptibility to poliomyelitis between portions of populations of different socioeconomic levels, and the differences demonstrated in the survey indicated that the nonrequesting population might well be a more resistant group than the inoculated.

The vaccination program was carried out between the end of April and mid-June. The first problem was that of collecting and verifying the basic information on each of the 1,829,996 children in the total study population. It included a record of the lot of material given in each inoculation to each child.

The second phase in the evaluation was concerned with discovery and identification of all cases of poliomyelitis or suspected poliomyelitis in all children of the first, second, and third grades in the study areas. The criteria for diagnosis of poliomyelitis, for designation of a case as paralytic and its severity, and for the interpretation of laboratory results were carefully drawn. The information concerning each case was carefully reviewed and the diagnostic classification made without knowledge of its vaccination status so that objectivity was readily maintained.

The study period was defined as the period from two weeks after the time of the third injections in an area to December 31, 1954. During the study period there were 1,013 cases reported to be poliomyelitis or suspected poliomyelitis: 428 in the total study population of placebo areas and 585 in the total study population of observed areas. The table shows the cases distributed according to the various segments of the total populations involved. It must be emphasized again that the evaluation is limited to a comparison of incidence in the vaccinated and in the established controls.

The nonparticipating portions of the populations, that is, those who specifically refused to participate, are not additional controls. That decision was firmly established well in advance of the tabulation of results. The data

Summary of Study Cases by Diagnostic Class & Vaccination Status, Rate per 100,000

Study Group	Study Population	All Reported Cases		Poliomyelitis Cases						Doubtful or not Poliomyelitis	
				Total		Paralytic		Non-paralytic			
	No.	*No.*	*Rate*	*No.*	*Rate*	*No.*	*Rate*	*No.*	*Rate*	*No.*	*Rate*
All areas, total	1,829,916	1,013	55	863	47	685	37	178	10	150	8
Placebo areas, total	749,236	428	57	358	48	270	36	88	12	70	9
Vaccinated	200,745	82	41	57	28	33	16	24	12	25	12
Placebo	201,229	162	81	142	71	115	57	27	13	20	10
Not inoculated*	338,778	182	54	157	46	121	36	36	11	25	7
Incomplete vaccinations	8,484	2	24	2	24	1	12	1	12	–	–
Observed areas, total	1,080,680	585	54	505	47	415	38	90	8	80	7
Vaccinated	221,998	76	34	56	25	38	17	18	8	20	9
Controls†	725,173	439	61	391	54	330	46	61	8	48	6
2nd grade not inoculated	123,605	66	53	54	44	43	35	11	9	12	10
Incomplete vaccinations	9,904	4	40	4	40	4	40	–	–	–	–

* Includes 8,577 children who received one or two injections of placebo.

† 1st and 3rd gra .e total population.

are presented only because they are available and for completeness of reporting. The cases and rates per 100,000 of specific population are listed. In the placebo control areas there was a total of 82 cases reported among the 200,745 vaccinated, and 162 among the 201,229 who received a placebo. The corresponding rates are 41 and 81—a ratio of 1:2.

The question has been asked why the incidence of paralytic cases is less in the uninoculated members of the population than in the placebo controls. One can only repeat that the populations receiving vaccine or placebo are strictly comparable in every characteristic; they are equal parts of one population, while those who refused participation are distinctly different.

Journal of the American Medical Association 158:1266-1268; August 6, 1955.

Guide to Paper by Thomas Francis, Jr.

1. *What were the differences between the two plans used to test the Salk vaccine?*
2. *What were the advantages and disadvantages of each plan?*
3. *Why weren't those who did not volunteer to participate in the second testing plan considered as an additional control group?*
4. *What do the data in Table I indicate about the effectiveness of the Salk vaccine?*
5. *What unexpected results about the rate of paralytic cases do the data reveal?*

Albert Sabin—Polio Immunity from a Drink

When Jonas Salk's paper on "Recent Studies on Immunization Against Poliomyelitis" was published in 1953, Albert B. Sabin (1906-) was completing nearly twenty years of virus research. In that same year, Sabin began his efforts to use attenuated (weakened) live polio virus to produce immunity.

Sabin was born in Russia to poor parents. When Albert was 15 the Sabin family emigrated to the United States. They settled in New Jersey where Albert attended high school. After graduating from college, he entered dental school at the request of an uncle who offered to pay his tuition. However, after reading the book *Microbe Hunters*, Sabin decided to leave dental school after completing two years there and to study medicine. He was now forced to work his way through medical school, as his uncle

withdrew his financial support. Like Salk, Sabin was more interested in research than in a medical practice. He worked at the Rockefeller Institute, where he began to look for strains of polio virus that were not harmful, but still capable of stimulating antibodies to produce immunity. It was during this period that he discovered that the polio virus affects the intestinal system. This discovery led him to think about an oral vaccine.

In 1939, Sabin became associated with the Children's Hospital in Cincinnati, Ohio. After tests on thousands of animals–10,000 monkeys and 160 chimpanzees, Sabin isolated the virus he was looking for. In 1956, he and his family were the first humans to drink the new vaccine. After a few small-scale tests, in which none of the subjects developed polio, and all showed higher levels of antibodies for the polio virus, the vaccine became ready for a mass testing program.

Incidents which occurred during the testing of the Salk vaccine made it difficult to test the Sabin vaccine, which used live virus, in the United States. Some children accidentally had received live polio virus instead of killed virus due to an error by a pharmaceutical company. In addition, there were too many children who had been already immunized by the Salk vaccine.

The Soviet Union agreed to field test the Sabin vaccine. In 1957 Russian children were given live virus orally with syrup or candy. By 1959, 4,500,000 people in the USSR, Poland, Czechoslovakia, Singapore, and Mexico were given the Sabin vaccine. By 1960, 15 million were tested. The results indicated almost complete immunization with no ill effects.

In the United States, medical authorities were slow to license the Sabin vaccine, as they feared the live virus aspect of the vaccine. In 1961 the Sabin vaccine was approved, and it is now used throughout the United States, being given to children before the age of one.

BEHAVIOR OF CHIMPANZEE-AVIRULENT POLIOMYELITIS VIRUSES IN EXPERIMENTALLY INFECTED HUMAN VOLUNTEERS

ALBERT B. SABIN (1955)

Poliomyelitis is generally more severe in adults than in very young children. Any method of immunization must avoid the possibility of creating a large

adult population without resistance to paralytic poliomyelitis. Natural infection in early childhood, the process by which the vast majority of the world population acquires immunity to poliomyelitis, is known to provide life-long protection. The remarkable observations on Eskimos indicate that even in the absence of reinfection the body continues to produce specific antibodies for as long as 40 years after exposure to the virus. Natural infection with poliomyelitis viruses thus seems to provide the body with a "built-in" booster mechanism.

Studies on experimentally produced and naturally occurring attenuated strains of poliomyelitis virus are designed to provide information which would indicate whether or not the natural process of immunization can be duplicated without incurring the varying risk of paralysis encountered in nature.

The purpose of the present study was to determine by quantitative methods the comparative behavior in human beings of the same virus culture fluids which had been extensively studied for a period of 18 months in monkeys and chimpanzees. The volunteers, 21 to 30 year old inmates of a federal reformatory, were selected on the basis of previous tests which showed that they lacked demonstrable antibody for one or more types of poliomyelitis virus.

The three types of virus in the form of monkey kidney tissue culture fluid were administered in doses of 0.001, 0.1, 0.5, or 1 c.c. in a teaspoonful of milk to 26 men; 8 men for the Type 1 virus, and 9 each for Types 2 and 3. Quantitative tests for virus were performed at frequent intervals on the stools and blood and on swabs from the mouth and tongue. Antibodies were determined quantitatively over a period of 3 months.

Results

The results of this study, which will be reported in detail elsewhere, may be summarized as follows:

1. Human beings are more susceptible than chimpanzees to infection by the oral route. The smallest virus dose used, sufficed to produce an immunogenic alimentary infection in the volunteers.

2. The antibody response was of a higher order in the volunteers with extensive multiplication of virus in the throat than in those who exhibited little or no multiplication in the throat. This finding suggests that a more highly immunogenic, and perhaps more localized, infection could be produced by simply swabbing the throat with virus, and experiments on this mode of infection are now in progress.

The American Journal of the Medical Sciences 230:1-8; July 1955.

PRESENT STATUS OF ATTENUATED LIVE VIRUS POLIOMYELITIS VACCINE

ALBERT B. SABIN (1957)

It may be said that much has been learned about the basic principles underlying immunization with attenuated polio viruses by the oral route, and that we now have strains derived from single particles of virus that are sufficiently highly attenuated and stable under appropriate conditions of cultivation to justify their use for the next stepwise studies on immunization of human beings. Experience has taught me the importance of accentuating the negative—and I therefore wish to stress that we are not ready for so-called mass trials of an oral vaccine but only for those tests on increasingly larger groups which must precede any consideration of tests on a large scale. I also want to accentuate the positive by saying that the Salk vaccine is the only polio vaccine available for public use at this time and that advantage should be taken of its protective effects to the maximum extent of its availability. I may add that some reservations that I had before disappeared with the demonstration that the antibodies and immunity produced by the Salk vaccine do not interfere with the alimentary infection produced by an oral attenuated vaccine or naturally acquired virus. This then is as far as we have gone, and it is obvious that we still have a great deal to learn before the ultimate goal of complete elimination of poliomyelitis is achieved.

attenuated—in a weakened, less powerful state
immunogenic—capable of producing immunity

In Bulletin of the New York Academy of Medicine 33:38; January 1957.

Guide to Papers by Albert B. Sabin

1. *What problem did Sabin attempt to solve?*
2. *Why was it necessary for Sabin to choose volunteers who did not possess antibodies for poliomyelitis virus?*
3. *How does Sabin's method for producing immunity to polio differ from Salk's method?*
4. *What is the significance of Sabin's first result?*
5. *What is the significance of Sabin's finding that in some volunteers there was "extensive multiplication of virus in the throat"?*
6. *Briefly summarize Sabin's feelings about the use of his vaccine and use of the Salk vaccine at the time of the second paper (1957).*

Jonas E. Salk—Two Decades of Polio Vaccine

More than twenty years have passed since the introduction of the first successful vaccine to prevent polio. Two possible approaches were considered. One involved a vaccine containing killed polio virus, as developed by Salk. The other, Sabin's vaccine, used live viruses whose ability to cause polio had been eliminated. There has always been controversy as to which of the two types of vaccine is the more effective. In the following article, Jonas Salk continues the debate.

POLIO: A CURE FOR THE NEW CONTROVERSY

JONAS E. SALK (1973)

SAN DIEGO—This is the twentieth anniversary of the announcement of the polio vaccine. A strange and paradoxical situation exists now in the United States with respect to immunization. Although the incidence of the disease has been sharply reduced, nevertheless cases continue to occur. The reasons for this are clear. So is the remedy. Why, then, do the authorities who are aware of the problem seem to be looking the other way?

From the *Journal of the American Medical Association*, October 1969: "Although poliomyelitis now occurs infrequently in the U.S., cases of paralytic illness occur in temporal association with administration of oral poliovirus vaccine."

From the *Weekly Reports*, U.S. Public Health Service Center for Disease Control, December 1972: "Although rare, the occurrence of paralytic poliomyelitis in contacts and recipients of oral poliovirus vaccine is a well-recognized phenomenon. In the ten-year period from 1961, when it was first licensed for use in this country, through 1971 . . . 109 vaccine-associated cases were reported. Thirty-six of these occurred in close contacts of vaccine recipients."

From the Annual Poliomyelitis Survey 1971, USPHS Center for Disease Control, March 1973: "Seventeen cases of paralytic poliomyelitis, with two deaths, were reported in the U.S., in 1971. . . . One case was 'recipient vacine-associated,' eight cases were 'contact vaccine-associated'."[1]

[1] This paragraph omitted from original publication; noted in *The Times*, June 9, 1973.

From the June 1972 Report of the Public Health Service Advisory Committee on Immunization Practices:

"Between 1955, when IPV [inactivated poliovirus vaccine—i.e., Salk-killed virus vaccine] was introduced, and 1962, when live, attenuated vaccine [Sabin oral vaccine] became widely used, more than 400 million doses of IPV were distributed in the U.S. Primary immunization with IPV plus regular booster doses provided a high degree of protection against paralytic diseases."

The authorities are aware that the live virus vaccine is responsible for some of the cases of polio which continue to occur in the United States and in at least nine other countries from which reports are available. This tendency of the oral vaccine to cause polio is inherent because of the genetic instability of the virus strains used. They are also aware that in Sweden, where only a killed virus vaccine has been used, polio has not occurred since 1966. In the same interval in the U.S., 192 cases of polio have been reported, a difference which is significant even though the population of Sweden is twenty times smaller.

The Advisory Committee Report makes the statement that the live virus vaccine "is easier to administer and produces antibody levels like that of the natural disease." This misleads one to believe that the immunity produced by the live virus vaccine is superior to that produced by the killed virus vaccine. However, this implication is not borne out by experience. The only advantage, then, that can be claimed for the live virus vaccine is its oral administration. Since all other vaccines have to be administered by injection or scarification, and since a killed virus vaccine has been shown to produce the desired effect without the risk of the vaccine itself causing polio, we might wonder why a toll of several cases of paralysis a year and an occasional death must be paid because it is "easier to administer" polio virus vaccine orally.

Ten years ago we reported that high and lasting antibody levels to the three types of polio virus could be induced in human subjects using a vaccine made of killed virus. The prevalent dogma then stated that a living virus vaccine would be necessary for effective control of polio. In light of the Swedish evidence this position is clearly untenable. Moreover, in some areas of the world, such as Africa and Latin America, the live virus vaccine is only partially effective because of the high prevalence of intestinal viruses which interfere with the establishment of the polio virus infection necessary for the oral vaccine's immunizing effect.

Because of the prevailing "live virus vaccine dogma," in 1961 the American Medical Association recommended that physicians in this country switch to the oral vaccine. Following this advice, use of the killed virus vaccine was gradually reduced to the point where it has now been completely replaced by the oral vaccine, and is no longer either manufactured or distributed in this country.

In retrospect, this changeover was both unnecessary and ill-advised. Had the switch not been made, and had the still-needed steps been taken to immunize the remaining pockets of unvaccinated individuals, the control of polio could have been as complete today in this country as it is in Sweden.

It is not too late to change back. However, those in authority seem reluctant even to make the facts known to the general public much less to consider the necessary changes. Even if present policies remain unchanged, the people of this country should at least have the same freedom of choice that exists in other countries, such as Canada and France, in which both live and killed virus vaccines are available.

In the absence of any other voices, I feel a responsibility to inform the public that they can justifiably demand that, if nothing else, the killed virus vaccine at least be made available in the United States so that they can request from their physicians a vaccine which is not only effective but completely safe.

"Polio: A Cure for the New Controversy." The New York Times New York. May 26, 1973. P. 31
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Guide to Paper by Jonas E. Salk

Analyze Salk's article in terms of what you have already studied about the struggle to develop a vaccine to prevent polio.

THE CONQUEST OF DISEASE–SUMMARY QUESTIONS

1. *Briefly describe the method of preventing disease used by each investigator in this section.*
2. *Which scientists in this section used similar methods to prevent disease?*
3. *Prepare a report on the conquest of a disease not included in this section.*

THE ORIGIN OF LIFE

Francesco Redi—A Simple Experiment Challenges a Widely Accepted Belief

Until the last half of the seventeenth century, the notion that living organisms could arise from nonliving matter was widely believed. According to this idea of spontaneous generation small animals and plants could be formed in dirt or mud and especially in decaying flesh. Shakespeare, in his play Hamlet, spoke of "the sun breeding maggots in dead dogs." It was thought that frogs and toads arose from the mud of ponds and streams with the help of the sun's rays; and bats were supposed to come from the river Nile.

This belief in spontaneous generation, which was so firmly implanted in the minds of the people of the time, was subjected to an experimental test by Francesco Redi (1626-1697). Redi was an Italian physician who was medical advisor to the grand dukes of Tuscany. In addition to his interest in science, Redi was known as a poet and man of literary talent. He differed from those naturalists of the period who believed that the Greek philosopher Aristotle's writings about spontaneous generation should not be questioned. No one before Redi had even attempted to test experimentally the theory of spontaneous generation. The following paper reports Redi's experiments.

EXPERIMENTS ON THE GENERATION OF INSECTS

FRANCESCO REDI (1668)

Although content to be corrected by any one wiser than myself, if I should make erroneous statements, I shall express my belief that the Earth, after having brought forth the first plants and animals at the beginning by order of the Supreme Creator, has never since produced any kinds of plants or

animals. Everything which we know in past or present times that she has produced, came solely from the true seeds of the plants and animals themselves, which thus preserve their species. And although it be a matter of daily observation that infinite numbers of worms are produced in dead bodies and decayed plants, I feel inclined to believe that these worms are all generated from other worms and that the decayed matter in which they are found has no other function than that of serving as a place, or suitable nest, where animals deposit their eggs at the breeding season, and in which they also find nourishment.

I began to believe that all worms found in meat were derived directly from the droppings of flies, and not from the decay of the meat. I was still more confirmed in this belief by having observed that, before the meat grew wormy, flies had hovered over it, of the same kind as those that later bred in it. Belief would be vain without the confirmation of experiment. In the middle of July, I put a snake, some fish, some eels and a slice of veal in four large, wide-mouthed flasks, having well closed and sealed them. I then filled the same number of flasks in the same way, only leaving these open. It was not long before the meat and the fish, in these second vessels, became wormy and flies were seen entering and leaving at will; but in the closed flasks I did not see a worm, though many days had passed since the dead flesh had been put in them. Outside on the paper cover there was now and then a deposit, or a maggot that eagerly sought some opening by which to enter and obtain nourishment. Meanwhile the different things placed in the flasks had become putrid and stinking.

Not content with those experiments, I tried many others at different seasons, using different vessels. In order to leave nothing undone, I even had pieces of meat put under ground, but though remaining buried for weeks, they never bred worms, as was always the case when flies had been allowed to light on the meat.

In Experiments on the Generation of Insects. Mab Bigelow, Editor. Open Court Publishing Company, Chicago, Illinois. 1909.

Guide to Paper by Francesco Redi

1. *What was Redi's hypothesis as to how new living things are produced?*
2. *What was Redi's hypothesis in explaining the appearance of worms on decaying meat?*
3. *Describe the experiment Redi designed to test this hypothesis.*
4. *What were his observations?*
5. *What additional efforts did Redi make to control his experiment?*

Lazaro Spallanzani—Setting the Stage for Pasteur

Lazaro Spallanzani (1724-1799), a countryman of Redi, became involved in the controversy over spontaneous generation, about seventy years after Redi's death. Spallanzani, the son of a lawyer, was trained to be a priest, studied law, and eventually followed a scientific career.

He was an excellent experimenter, studying the circulation of the blood, respiration, digestion, and regeneration. Spallanzani also was able to determine that sperm cells were necessary for animal fertilization. In 1765 he became aware of the work of the English biologist, John Needham, who reported data which seemed to support the teaching of spontaneous generation of microorganisms.

Spallanzani decided to repeat Needham's experiments under more rigorous conditions. He was able to create an airtight seal for his vessels by using an air pump. Needham, on the other hand, had used only corks to cover his vessels.

Although Spallanzani's experiment provided information which cast doubt upon the idea of spontaneous generation, it was not until the experiments of Louis Pasteur that this idea was finally proved false.

OBSERVATIONS AND EXPERIMENTS UPON THE MICROORGANISMS OF INFUSIONS

LAZARO SPALLANZANI (1799)

The idea, that microorganisms come from the air, appears to me to be confirmed by undoubted facts. I took sixteen large and equal glass vases; four I sealed hermetically (air tight); four were stopped with a wooden stopper, well fitted; four with cotton; and the four last I left open. In each of the four classes of vases, were hempseed, rice, lentils, and peas. The infusions were boiled a full hour, before being put into the vases. I began the experiments 11 May and visited the vases 5 June. In each there were two kinds of microorganisms, large and small; but in the four open ones, they were so numerous and confused that the infusions, if I may use the expression, rather seemed to teem with life. In those stoppered with cotton, they were about a third more rare; still fewer in those with wooden stoppers; and much more so in those hermetically sealed.

The number of microorganisms developed is proportioned to the communication with the external air. The air either conveys the germs to the infusions, or assists the expansion of those already there.

"Tracts on the Nature of Animals and Vegetables."
Translated by J. G. Dalyell, Edinburgh, 1799.

Guide to Paper by Lazaro Spallanzani

1. *What hypothesis was Spallanzani testing?*
2. *What method did Spallanzani use to test his hypothesis?*
3. *What were his observations?*
4. *What were Spallanzani's conclusions?*
5. *How did Spallanzani's work extend the work done by Redi to disprove spontaneous generation?*

Louis Pasteur—The End of Spontaneous Generation

How do new living things arise? Can they be formed spontaneously from dead material, or are living things produced only by other living things?

The work of Redi—with which you are familiar—convinced scientists that there was no spontaneous generation of living things which are large enough to be seen with the naked eye. However, spontaneous generation was still used to explain the origin of microscopic organisms.

Spallanzani attempted to show that even these microorganisms were not produced by spontaneous generation. His results did not convince all of the scientific world for two reasons: First, his method of boiling the infusion might have destroyed a "vegetative force" which was needed in order for spontaneous generation to take place. Second, by making his vessels airtight, Spallanzani might have eliminated any air required in the process of spontaneous generation.

About one hundred years after Spallanzani's experiment, the great French scientist Louis Pasteur (1832-1895) entered into the controversy. He used his creative ability to design an experiment which eliminated the objections that were raised against the work

of other scientists who had attempted to disprove the theory of spontaneous generation. Therefore, finally, in the year 1861, Louis Pasteur was able to lay this theory to rest.

ON THE ORGANIZED BODIES WHICH EXIST IN THE ATMOSPHERE; EXAMINATION OF THE DOCTRINE OF SPONTANEOUS GENERATION

LOUIS PASTEUR (1861)

I believe I have rigorously established that all microorganisms in infusions which have been previously heated originate only from the solid particles carried by the air and which are constantly being deposited on all objects. In order to remove the slightest doubt in the reader, may I present the results of the following experiments.

In a glass flask I placed one of the following liquids which are extremely alterable through contact with ordinary air: yeast water, sugared yeast water, urine, sugar beet juice, pepper water. Then I drew out the neck of the flask under a flame, so that a number of curves were produced in it. I then boiled the liquid for several minutes until steam issued freely through the extremity of the neck. This end remained open without any other precautions. The flasks were then allowed to cool. Any one who is familiar with the delicacy of experiments concerning the so-called "spontaneous" generation will be astounded to observe that the liquid treated in this casual manner remains indefinitely without alteration. The flasks can be handled in any manner, can be transported from one place to another, can be allowed to undergo all the variations in temperature of the different seasons, the liquid does not undergo the slightest alteration. It retains its odor and flavor. In no case is there the development of microorganisms in the liquid.

It might seem that atmospheric air, entering with force during the first moments, might come in contact with the liquid in its original crude state. This is true, but it meets a liquid which is still close to the boiling point. The further entrance of air occurs much slower, and when the liquid has cooled to the point where it will not kill the germs, the entrance of air has slowed down enough so that the dust it carries which is able to act on the infusion and cause the development of microorganisms is deposited on the moist walls of the curved tube. At least, I can see no other explanation for these curious results. For, after one or more months in the incubator, if the neck of the flask is removed by a stroke of a file, without otherwise touching the flask, molds and infusoria begin to appear after 24, 36, or 48 hours, just as usual, or as if dust from the air had been inoculated into the flask.

The same experiment can be repeated with milk, except here one must take the precaution of boiling the liquid under pressure at a temperature above 100° and allowing the flask to cool with the reentry of heated air. The flask can be allowed to stand in the open, just as before. The milk undergoes no alteration. I have allowed milk prepared in this manner to incubate for many months at 25 to 30°, without alteration.

I do not know any more convincing experiments than these, which can be easily repeated and varied in a thousand ways . . .

At this moment I have in my laboratory many highly alterable liquids which have remained unchanged for 18 months in open vessels with curved or inclined necks. A number of these were deposited in the office of the Academy of Sciences during the meeting of 6 February 1860, when I had the honor of communicating to them these new results.

The great interest of this method is that it proves without doubt that the origin of life, in infusions which have been boiled, arises uniquely from the solid particles which are suspended in the air.

infusion–a soup-like liquid prepared by boiling materials in water

Annales des Sciences Naturelles. 4th Series. Vol. 16. Pp. 5-98. 1861. In Milestones in Microbiology. T. D. Brock, Translator and Editor. Prentice-Hall, Inc., Englewood Cliffs, New Jersey. 1961.

Guide to Paper by Louis Pasteur

1. *According to Pasteur, what is the origin of microorganisms found in infusions?*
2. *Describe Pasteur's method by which he verified his idea.*
3. *What were Pasteur's observations about the liquid in the flask?*
4. *Under what conditions did he find microorganisms in the flask?*
5. *How did the shape of the flask help Pasteur verify his theory?*
6. *Describe how Pasteur's experiment improved upon Spallanzani's work.*

Stanley L. Miller—Returning to the Theory of Spontaneous Generation

We have seen how the experiments of Redi, Spallanzani, and, finally Pasteur eliminated the idea of spontaneous generation from biological thought. The key idea established from their work was that living things can be produced only from other living things.

Some of you may wonder, "Where did the *very first* living things on earth come from? Twenty-five years after the death of Pasteur, the Russian scientist Oparin suggested that amino acids, carbohydrates, and other important compounds were present on the earth before living things arose, and were needed for the first living things to be formed.

According to Oparin's hypothesis, these organic compounds came together to form larger, more complex groupings of compounds. Gradually, these larger groupings gave rise to living things.

But where did these basic building blocks of life (the amino acids, carbohydrates, etc.) come from?

Stanley Miller (1930-) designed an experiment to show how amino acids could have been formed on the primitive earth. Miller was born in California, where he completed his undergraduate degree. He continued his scientific training at the University of Chicago. In working for his PhD, Miller was influenced by one of his professors, Harold Urey, a Nobel Prize winner in chemistry. Urey believed that larger organic compounds could be made from smaller elements and compounds through electric discharges. Miller tested his professor's idea.

It seems that Miller's findings reopen the hypothesis of spontaneous generation as a possible explanation for the origin of life.

A PRODUCTION OF AMINO ACIDS UNDER POSSIBLE PRIMITIVE EARTH CONDITIONS

STANLEY L. MILLER (1953)

The idea that the organic compounds that serve as the basis of life were formed when the earth had an atmosphere of methane, ammonia, water, and hydrogen instead of carbon dioxide, nitrogen, oxygen, and water was suggested by Oparin and has been given emphasis recently by Urey and Bernal.

In order to test this hypothesis, an apparatus was built to circulate CH_4, NH_3, H_2O, and H_2 past an electric discharge. The resulting mixture has been tested for amino acids by paper chromatography.

The experimental procedure was to seal off the opening in the boiling flask

after adding 200 ml of water, evacuate the air, add 10 cm pressure of H_2, 20 cm of CH_4, and 20 cm of NH_3. The water in the flask was boiled, and the discharge was run continuously for a week. At the end of the run the solution in the flask was removed and 1 ml of mercuric chloride added to prevent the growth of living organisms.

The discharge, a small corona, was provided by an induction coil designed for detection of leaks in vacuum apparatus.

A paper chromatogram (of the mixture from the electric discharge apparatus) was run in *n*-butanol-acetic-acid–water mixture followed by water-saturated phenol, and spraying with ninhydrin. Identification of an amino acid was made when the R_f value (the ratio of the distance traveled by the amino acid to the distance traveled by the solvent front), the shape, and the color of the spot were the same on a known, unknown, and mixture of the known and unknown; and when consistent results were obtained with chromatograms using phenol and 77% ethanol.

On this basis glycine, α-alanine and β-alanine are identified. The identification of the aspartic acid and α-amino-*n*-butyric acid is less certain because the spots are quite weak. The spots marked A and B are unidentified as yet, but may be beta and gamma amino acids. These are the main amino acids present, and others are undoubtedly present but in smaller amounts. It is estimated that the total yield of amino acids was in the milligram range.

In this apparatus an attempt was made to duplicate a primitive atmosphere of the earth, and not to obtain the best conditions for the formation of amino acids. Although in this case the total yield was small for the energy expended, it is possible that, with more efficient apparatus this type of process would be a way of commercially producing amino acids. Electrical discharge, therefore may have played a significant role in the formation of compounds in the primitive atmosphere.

Science 117:528-529; May 15, 1953.

Guide to Paper by Stanley L. Miller

1. *What hypothesis was Miller testing?*
2. *Describe the procedure that Miller used to test this hypothesis.*
3. *What results did Miller obtain through the use of paper chromatography?*
4. *How did Miller make certain that any amino acids formed were not produced by living organisms?*
5. *How does Miller's work contribute to the understanding of how life may have originated on the earth?*
6. *Contrast the explanations of the origin of life as revealed by the papers of Redi and Miller.*

THE ORIGIN OF NEW ORGANISMS

Jean Lamarck—An Early Theory of Evolution

Of all the areas in the study of biology, the field of evolution has probably caused the greatest debate. The mystery of how new living things have developed from earlier simpler forms has puzzled scholars since the days of the ancient Greek philosopher Aristotle. During the first half of the nineteenth century a new view of evolution was advanced by a French biologist, Jean Lamarck (1744-1829)

Lamarck joined the French army at the age of 17 after studying under the Jesuits. After the war, he went to Paris to study medicine, working as a bank clerk in order to support himself. He became interested in botany and published a classification system of French plants. In 1781 he became botanist to King Louis XVI, a position he held until 1794. Then at the age of 50, Lamarck turned his attention from the study of plants to the study of animals.

His first undertaking as a zoologist was to organize the available knowledge concerning invertebrates. Slowly Lamarck began to question the popularly held belief that species did not change. He developed a theory to explain how changes have taken place in animals over long periods of time.

Lamarck received little recognition during his lifetime. He lived in poverty, supporting a large family, and became blind in his later years. It was through the devotion of his daughter that Lamarck was able to continue his work. Her prophecy, "Posterity will honor you," was realized many years later when Lamarck's theory served as a basis for the development of a modern theory of evolution.

ZOOLOGICAL PHILOSOPHY

JEAN LAMARCK (1809)

The environment affects the shape and organization of animals, that is to say that when the environment becomes very different, it produces in course of time corresponding modifications in the shape and organization of animals.

If a new environment, which has become permanent for some race of animals, induces new habits in these animals, that is to say, leads them into new activities which become habitual, the result will be the use of some one part in preference to some other part, and in some cases the total disuse of some part no longer necessary.

Nothing of all this can be considered as hypothesis or private opinion; on the contrary, they are truths which, in order to be made clear, only require attention and the observation of facts.

Snakes have adopted the habit of crawling on the ground and hiding in the grass; so that their body, as a result of continually repeated efforts at elongation for the purpose of passing through narrow spaces, has acquired a considerable length, quite out of proportion to its size. Now, legs would have been quite useless to these animals and consequently unused. Long legs would have interfered with their need of crawling, and very short legs would have been incapable of moving their body, since they could only have had four. The disuse of these parts thus became permanent in the various races of these animals, and resulted in the complete disappearance of these same parts, although legs really belong to the plan or organization of the animals of this class.

The frequent use of any organ, when confirmed by habit, increases the functions of that organ, leads to its development, and endows it with a size and power that it does not possess in animals which exercise it less.

We have seen that the disuse of any organ modifies, reduces, and finally extinguishes it. I shall now prove that the constant use of any organ, accompanied by efforts to get the most out of it, strengthens and enlarges that organ, or creates new ones to carry on the functions that have become necessary.

The bird which is drawn to the water by its need of finding there the prey on which it lives, separates the digits of its feet in trying to strike the water and move about on the surface. The skin which unites these digits at their base acquires the habit of being stretched by these continually repeated separations of the digits; thus in course of time there are formed large webs which unite the digits of ducks, geese, etc. as we actually find them.

It is interesting to observe the result of habit in the peculiar shape and size of the giraffe; this animal, the largest of the mammals, is known to live in the interior of Africa in places where the soil is nearly always arid and barren, so that it is obliged to browse on the leaves of trees and to make constant efforts to reach them. From this habit long maintained in all its race, it has resulted that the animal's fore-legs have become longer than its hind legs, and that its neck is lengthened to such a degree that the giraffe, without standing up on its hind legs, attains a height of six metres (nearly twenty feet).

Philosophie Zoologique Paris. 1809. Translated by H. Elliott. Macmillan Company, London. 1914.

Guide to Paper by Jean Lamarck

1. *Summarize Lamarck's ideas as to the origin of a new species.*
2. *Criticize the following statement of Lamarck: "Nothing of all this can be considered as hypothesis or private opinion; on the contrary, they are truths which, in order to be made clear, only require attention and the observation of facts."*
3. *Can you suggest one or more possible explanations for the observed facts other than the one put forth by Lamarck?*

Charles Darwin—From a Voyage to a Theory

What helps make a person become world famous? Is it luck and chance, or is it background and training? In the case of Charles Darwin (1809-1882), it was probably a combination of these factors which brought him international recognition.

He was born in England, the son and grandson of medical men. At the age of 16, Darwin, continuing in the family tradition, went to Scotland to study medicine. After a while, he decided that he was not interested in the medical profession. Darwin couldn't stand the sight of blood, and he found the anatomy lectures very boring.

His father then sent him to Cambridge University for training as a clergyman, but he was not an outstanding student.

Darwin's main interest was in collecting insects, rocks, flowers, and other kinds of specimens. This hobby was encouraged by one of his professors, who later recommended him for the position of naturalist on a ship. This ship, "H.M.S. Beagle," was sent mainly to survey the West Coast of South America.

The voyage of the "Beagle" lasted five years, from 1831-1836. During this time, Darwin took long trips on shore where he made observations and collected samples. He noticed the similarities between animals found on the islands and those found on the mainland 600 miles away. On the Galapagos Islands located off the coast of Ecuador, Darwin began to wonder about the origin of the different kinds of plants and animals that he saw. Each separate island had its own species of birds. It didn't seem logical to Darwin that a separate species was created for each small island.

Why these birds were similar to those found in South America also puzzled him.

After the voyage, Darwin returned to England, and for more than twenty years he studied the specimens and notes he collected during his stay on the "Beagle."

In 1858, Darwin, who had been gathering facts for his theory of evolution and had been testing it experimentally, was surprised to find that Alfred Wallace had independently come to the same conclusions. Darwin decided to report his work and Wallace's work in a joint presentation to the Scientific Society in London.

One year later, Darwin published his own great work, *On the Origin of Species by Means of Natural Selection.* The entire edition was sold out during the first day of publication. He created great controversy and debate. From the last half of the nineteenth century through modern times, Darwin's theory has been both accepted and attacked by different groups of people. Until recently, several states in the United States banned the teaching of Darwin's theory of evolution.

THE ORIGIN OF SPECIES BY MEANS OF NATURAL SELECTION

CHARLES DARWIN (1859)

When on board H.M.S. Beagle, as naturalist, I was much struck with certain facts in the distribution of the organic beings inhabiting South America, and in the geological relations of the present to the past inhabitants of that continent. These facts, seemed to throw some light on the origin of species—that mystery of mysteries, as it has been called by one of our greatest philosophers. On my return home, it occurred to me, in 1837, that something might perhaps be made out of this question by patiently accumulating and reflecting on all sorts of facts which could possibly have any bearing on it. After five years' work I allowed myself to speculate on the subject, and drew up some short notes; these I enlarged in 1844 into a sketch of the conclusions, which then seemed to me probable: from that period to the present day I have steadily pursued the same object. I hope that I may be excused for entering on these personal details, as I give them to show that I have not been hasty in coming to a decision.

My work is now (1859) nearly finished; but as it will take me many more

years to complete it, and as my health is far from strong, I have been urged to publish this abstract. I have more especially been induced to do this, as Mr. Wallace, who is now studying the natural history of the Malay Archipelago, has arrived at almost exactly the same general conclusions that I have on the origin of species.

In considering the origin of species, it is quite conceivable that a naturalist, reflecting on the mutual affinities of organic beings, on their embryological relations, their geographical distribution, geological succession, and other such facts, might come to the conclusion that species had not been independently created, but had descended, like varieties, from other species. Nevertheless, such a conclusion, even if well founded, would be unsatisfactory, until it could be shown how the innumerable species inhabiting this world have been modified, so as to acquire that perfection of structure and coadaptation which justly excites our admiration. Naturalists continually refer to external conditions, such as climate, food, etc., as the only possible cause of variation. In one limited sense, as we shall hereafter see, this may be true; but it is preposterous to attribute to mere external conditions the structure, for instance, of the woodpecker, with its feet, tail, beak, and tongue, so admirably adapted to catch insects under the bark of trees. In the case of the mistletoe, which draws its nourishment from certain trees, which has seeds that must be transported by certain birds, and which has flowers with separate sexes absolutely requiring the agency of certain insects to bring pollen from one flower to the other, it is equally preposterous to account for the structure of this parasite, with its relations to several distinct organic beings, by the effects of external conditions, or of habit, or of the volition of the plant itself.

It is, therefore, of the highest importance to gain a clear insight into the means of modification and coadaptation. At the commencement of my observations it seemed to me probable that a careful study of domesticated animals and of cultivated plants would offer the best chance of making out this obscure problem. Nor have I been disappointed; in this and in all other perplexing cases I have invariably found that our knowledge, imperfect though it be, of variation under domestication, afforded the best and safest clue. I may venture to express my conviction of the high value of such studies, although they have been very commonly neglected by naturalists.

Although much remains obscure, and will long remain obscure, I can entertain no doubt, after the most deliberate study and dispassionate judgement of which I am capable, that the view which most naturalists until recently entertained, and which I formerly entertained—namely, that each species has been independently created—is erroneous. I am fully convinced that species are not immutable; but that those belonging to what are called the same genera are lineal descendants of some other and generally extinct

species, in the same manner as the acknowledged varieties of any one species are the descendants of that species. Furthermore, I am convinced that natural selection has been the most important, but not the exclusive, means of modification.

On the Origin of Species. John Murray, London. 1859.

Guide to Paper by Charles Darwin

1. *What led Darwin to wonder about the origin of the species?*
2. *What did Darwin feel it necessary for him to do in order to answer the question of the origin of the species?*
3. *What was Darwin's hypothesis as to the origin of the species?*
4. *What needed to be demonstrated to support Darwin's hypothesis?*
5. *How did Darwin attempt to find out how modification of species is accomplished?*
6. *How do we view Darwin's theory today?*

Alfred Russel Wallace—In Darwin's Shadow

Alfred Wallace (1823-1913) was born in a small English town. He did not enter college and worked as a land surveyor and architect. Wallace later became an English teacher at a small school, where a friend helped him develop an interest in collecting plants and insects. In 1848, he made an expedition to the Amazon river in Brazil in order to collect scientific material. Several years later he toured the Malay Islands. While studying the animals on these islands, Wallace found himself faced with the same problems as faced Darwin in the Galapagos Islands—why did each island have its own kind of animals?

For three years Wallace constantly thought about this problem. One night in 1858, while in the midst of an attack of tropical fever, he developed a theory. Two nights later his theory was completely written up and mailed to Charles Darwin in London. Darwin said upon reading Wallace's paper, "I never saw a more striking coincidence. If Wallace had my manuscript sketch written out in 1842, he could not have made a better short abstract! Even his terms now stand as heads of my chapters."

Darwin received most of the credit for the theory of evolution although both he and Wallace had independently come to the same conclusions. Wallace was not upset by Darwin's fame. In 1870 Wallace said, "I have felt all my life, and I still feel, the most sincere satisfaction that Mr. Darwin had been at work long before me, and that it was not left for me to write *The Origin of Species.*

ON THE TENDENCY OF VARIETIES TO DEPART INDEFINITELY FROM THE ORIGINAL TYPE

ALFRED RUSSEL WALLACE (1858)

The Struggle for Existence

The life of wild animals is a struggle for existence. The full exertion of all their faculties and all their energies is required to preserve their own existence and provide for that of their infant offspring. The possibility of procuring food during the least favorable seasons and of escaping the attacks of their most dangerous enemies are the primary conditions which determine the existence both of individuals and of entire species.

The numbers that die annually must be immense; and as the individual existence of each animal depends upon itself, those that die must be the weakest—the very young, the aged, and the diseased—while those that prolong their existence can only be the most perfect in health and vigor, those who are best able to obtain food regularly and avoid their numerous enemies. It is "a struggle for existence," in which the weakest and least perfectly organized must always succumb.

Useful Variations Will Tend to Increase, Unuseful or Hurtful Variations to Diminish

Most or perhaps all the variations from the typical form of a species must have some definite effect, however slight, on the habits or capacities of the individuals. Even a change of color might, by rendering them more or less distinguishable, affect their safety; a greater or less development of hair might modify their habits. More important changes, such as an increase in the power or dimensions of the limbs or any of the external organs, would more or less affect their mode of procuring food or the range of country which they could inhabit. It is also evident that most changes would affect, either favorably or adversely, the powers of prolonging existence. An antelope with shorter or

weaker legs must necessarily suffer more from the attacks of the feline carnivora; the passenger pigeon with less powerful wings would sooner or later be affected in its powers of procuring a regular supply of food; and in both cases the result must necessarily be a diminution of the population of the modified species.

If, on the other hand, any species should produce a variety having slightly increased powers of preserving existence, that variety must inevitably in time acquire a superiority in numbers.

Lamarck's Hypothesis Very Different from That Now Advanced

The hypothesis of Lamarck—that progressive changes in species have been produced by the attempts of animals to increase the development of their own organs and thus modify their structure and habits—has been repeatedly and easily refuted by all writers on the subject of varieties and species.

The giraffe did not acquire its long neck by desiring to reach the foliage of the more lofty shrubs and constantly stretching its neck for the purpose, but because any varieties which occurred among its ancestors with a longer neck than usual at once secured a fresh range of pasture over the same ground as their shorter-necked companions, and on the first scarcity of food were thereby enabled to outlive them.

Journal of the Proceedings of the Linnean Society
3: 45; August 1858. London.

Guide to Paper by Alfred Russel Wallace

1. *What does Wallace mean when he says: "The life of wild animals is a struggle for existence?"*
2. *According to Wallace, what effect do variations have on living things?*
3. *How does Wallace's explanation of how the giraffe acquired its long neck differ from Lamarck's?*
4. *Which of Wallace's ideas are similar to Darwin's?*

THE TRANSFER OF TRAITS

Gregor Mendel—Fame after Death

Imagine working eight years on a series of experiments, publishing the results and then having no one in the scientific community pay any attention to them. Such was the fate of Gregor Mendel (1822-1884). Born in Austria, the son of peasant farmers, Mendel showed an early liking for science. His parents had a strong desire to educate their son and also developed within him an interest in fruit growing.

After completing a scientific education, Mendel entered an Augustinian monastery. At the same time he also taught natural science in the high school of Brünn, the town in which the monastery was located.

In the garden of his monastery, Mendel, during his free time, conducted experiments to determine how traits are inherited in pea plants. What Mendel did was to cross (mate) different kinds of pea plants. He observed the traits of each new plant and recorded his results. Quite frequently he would invite his students to visit his garden. In 1865, he published his work in the *Journal of the Natural Science Society of Brünn*, and he attracted no attention, either in Brünn, or elsewhere. Several reasons have been suggested as to why this important paper went unnoticed. The journal in which he published was a local one and not widely known. It was a difficult paper and used new terms that scientists were not familiar with. In addition, naturalists at that time were preoccupied with questions raised by Darwin's Theory of Evolution published only a few years earlier.

After this publication, Mendel continued his experiments, until 1868, when he was elected abbot of his monastery. These duties prevented further scientific inquiry, and Mendel administered the monastery until his death in 1884.

Thirty-five years after Mendel's paper had been published, three investigators, working independently of one another, in three different countries, obtained results similar to his. In searching the

scientific literature for any data confirming these new experiments, they each came across Mendel's paper. Not only did Mendel publish the experimental data, but he also furnished the correct interpretations. With this "rediscovery" of Mendel's work, the new science of genetics was born.

EXPERIMENTS IN PLANT HYBRIDIZATION

GREGOR MENDEL (1865)

The paper now presented records the results of a detailed experiment. This experiment was practically confined to a small plant group, and is now, after eight years' pursuit, concluded in all essentials. Whether the plan upon which the separate experiments were conducted and carried out was the best suited to attain the desired end is left to the friendly decision of the reader.

Selection of the Experimental Plants

The value and utility of any experiment are determined by the fitness of the material for the purpose for which it is used, and thus in the case before us it cannot be immaterial what plants are subjected to experiment and in what manner such experiments are conducted.

The selection of the plant group which shall serve for experiments of this kind must be made with all possible care if it be desired to avoid from the outset every risk of questionable results.

The experimental plants must necessarily:

1. Possess constant differentiating traits
2. The hybrids of such plants must, during the flowering period, be protected from the influence of all foreign pollen, or be easily capable of such protection
3. The hybrids and their offspring should suffer no marked disturbance in their fertility in the successive generations.

In all, thirty-four more or less distinct varieties of Peas were obtained from several seedsmen and subjected to a two years' trial. For fertilization twenty-two of these were selected and cultivated during the whole period of the experiments.

Each two of the differentiating traits were united by cross-fertilization. There were made for the

1st trial 60 fertilizations on 15 plants
2nd trial 58 fertilizations on 10 plants
3rd trial 35 fertilizations on 10 plants
4th trial 40 fertilizations on 10 plants
5th trial 23 fertilizations on 5 plants
6th trial 34 fertilizations on 10 plants
7th trial 37 fertilizations on 10 plants

From a larger number of plants of the same variety only the most vigorous were chosen for fertilization. Weakly plants always afford uncertain results.

Furthermore, in all the experiments reciprocal crossings were effected in such a way that each of the two varieties which in one set of fertilization served as seed-bearer, in the other set was used as the pollen plant.

The Generation(Bred) from the Hybrids

In this generation there reappear, together with the dominant characters, also the recessive ones with their peculiarities fully developed, and this occurs in the indefinitely expressed average proportion of three to one, so that among each four plants of this generation three display the dominant character and one the recessive. This relates without exception to all the characters which were investigated in the experiments. The relative numbers which were obtained for each pair of differentiating characters are as follows:

Expt. 1. Form of seed–From 253 hybrids 7,324 seeds were obtained in the second trial year. Among them were 5,474 round or roundish ones and 1,850 angular wrinkled ones. Therefrom the ratio 2.96 to 1 is deduced.

Expt. 2. Colour–258 plants yielded 8,203 seeds, 6,022 yellow, and 2,001 green; their ratio, therefore, is as 3.01 to 1.

In the Proceedings of the Natural History Society of Brunn. February 8, and March 8, 1865. Translated by W. Bateson, "Mendel's Principles of Heredity," Cambridge, 1909.

Guide to Paper by Gregor Mendel

1. *Why did Mendel select plants that "possess constant differentiating traits?"*
2. *Why was it necessary for the plants to be protected from the influence of all foreign pollen?*
3. *Why was it necessary for the plants to "suffer no marked disturbance in their fertility?"*

4. *In mating tall plants with short plants, Mendel found that how the trait was inherited did not depend on whether the tall or the short plant supplied the pollen. How did he do this?*
5. *What did Mendel predict would be true about every four offspring produced from the mating of two hybrids?*

Hermann J. Muller—The Effects of X-Rays on Heredity

In 1909, at Columbia University, a professor of biology was telling a sophomore class about the rediscovery of Mendel's paper reporting his experiments with pea plants. One of the students listening was Hermann J. Muller (1890-1966), the only son of one of New York's leading makers of metal objects of art.

This student soon developed a great desire to follow a career in research related to heredity. Muller showed an early interest in science. While in high school, he helped start what may have been the first high school science club in the United States. He then majored in zoology at Columbia, where he was one of the founders of the Biology Club there. During summers he worked as a tutor, a bank clerk, and at a resort hotel. In 1915, he completed his academic work at Columbia, receiving a PhD.

Muller then became an instructor at Rice Institute in Texas, where he began his research into the process of heredity. He experimented on fruit flies, finally publishing his results in 1927.

Muller's work took him to the Institute of Genetics in Moscow, from 1933 to 1937. He left Russia when the Soviet Union began to imprison, banish, or execute many of its leading geneticists.

In the years before his death, Dr. Muller was engaged in a highly publicized campaign to warn the world that radiation from nuclear bomb tests could cause many harmful mutations in future generations. He also petitioned for all nations to abandon war because the hydrogen bombs "threatened the continued existence of mankind."

These actions, and others, made Dr. Muller a controversial figure. However, his major scientific achievements were never overlooked. He received many honors and awards during his

lifetime, including the Nobel Price in 1946 for his experiments on the hereditary effects of radiation.

ARTIFICIAL TRANSMUTATION OF THE GENE

HERMANN J. MULLER (1927)

Most modern geneticists will agree that gene mutations form the chief basis of organic evolution, and therefore of most of the complexities of living things. Unfortunately for the geneticists, however, the study of these mutations, and, through them, of the genes themselves, has heretofore been very seriously hampered by the extreme infrequency of their occurrence under ordinary conditions, and by the general unsuccessfulness of attempts to modify decidedly, and in a sure and detectable way this sluggish "natural" mutation rate.

It has been repeatedly reported that mutations could be induced by X or radium rays, but, as in the case of the similar published claims involving other agents (alcohol, lead, antibodies, etc.), the work has been done in such a way that the meaning of the data, as analyzed from a modern genetic standpoint, is not clear. Moreover, what were apparently the clearest cases have given negative or contrary results on repetition. Nevertheless, on theoretical grounds, it has appeared to the present writer that radiations of short wave length should be especially promising for the production of mutational changes, and for this and other reasons a series of experiments concerned with this problem has been undertaken during this past year on the fruit fly, *Drosophila Melanogaster,* in an attempt to provide critical data. The well-known favorableness of this species for genetic study, and the special methods evolved during the writer's eight years' intensive work on its mutation rate have finally made possible the finding of some decisive effects due to the application of X-rays.

It has been found quite conclusively that treatment of the sperm with relatively heavy doses of X-rays induces the occurrence of true "gene mutations" in a high proportion of the treated germ cells. Several hundred mutants have been obtained in this way in a short time and considerably more than a hundred of the mutant genes have been followed through these four or more generations. They are (nearly all of them, at any rate) stable in their inheritance, and most of them behave in the manner typical of the Mendelian chromosomal mutant genes found in organisms generally. Thousands of untreated parent flies were bred as controls in the same way as the treated ones. Comparison of the mutation rates under the two sets of conditions showed that the heavy treatment had caused a rise of about fifteen thousand percent in the mutation rate over that in the untreated germ cells.

The action of X-rays on the genes is not confined to the sperm cells, for treatment of the unfertilized females causes mutations about as readily as treatment of the males.

Among the visible mutations found, the great majority were recessive, yet there was a considerable "sprinkling" of dominants, just as in other work. All in all, then, there can be no doubt that many, at least, of the changes produced by X-rays are of just the same kind as the "gene mutations" which are obtained, with so much greater rarity, without such treatment, and which we believe furnish the building blocks of evolution.

Science 66:84-87; 1927.

Guide to Paper by Hermann J. Muller

1. *What problem faced geneticists who attempted to study mutations?*
2. *How have geneticists tried to solve the above problem?*
3. *Why did Muller feel that more investigation was needed in this area?*
4. *What was Muller's experiment?*
5. *What were his results?*
6. *What control did Muller use?*
7. *Why is Muller's work important?*

George W. Beadle and Edward L. Tatum— Using Bread Molds to Discover Gene Action

From the time of the rediscovery of Mendel's paper in 1900, biologists attempted to discover the nature of the gene. Throughout the 1920s and '30s scientists found that genes control specific traits. How the genes acted was still unknown.

George W. Beadle (1904-) studied the genetics of corn at Cornell University where he received his PhD in 1931. He continued his studies at Cal Tech where he investigated heredity in the fruit fly. After three years in California Beadle realized that his corn and fruit fly studies were not providing answers to the questions on how the genes act.

He then went to France where he developed a hypothesis of gene action in a laboratory in Paris. What was now needed was the experimental evidence to support his hypothesis. After many unsuccessful attempts to obtain this evidence, Beadle returned to

the United States, where he teamed up with the young chemist Edward L. Tatum in 1937.

By 1940 the investigators decided that they had to find a better organism than the fruit fly in order to study gene action. Beadle and Tatum turned their attention to the red bread mold *Neurospora*. Its life cycle is brief, it can be grown in vast quantities in the laboratory, and its genetics is well known. The following paper describes their experiments with this new organism.

In 1941 Beadle and Tatum published their results. The stage was now set for another pair of investigators to describe the structure of the gene. Beadle and Tatum were awarded the Nobel Prize in 1958.

GENETIC CONTROL OF BIOCHEMICAL REACTIONS IN NEUROSPORA

GEORGE W. BEADLE AND EDWARD L. TATUM (1941)

The development and functioning of an organism consists essentially of an integrated system of chemical reactions controlled in some manner by genes. It is entirely reasonable to suppose that these genes control or regulate specific reactions either by acting directly as enzymes or by determining how enzymes act.

We have set out to determine if and how genes control known biochemical reactions. *Neurospora* offers many advantages for such an approach and is well suited to genetic studies. Accordingly, our program has been built around this organism. The procedure is based on the assumption that X-ray treatment will induce mutations in genes concerned with the control of known specific chemical reactions. If the organism must be able to carry out a certain chemical reaction to survive on a given culture medium, a mutant unable to do this will obviously die on this medium. Such a mutant can be maintained and studied, however, if it will grow on a medium to which has been added the essential product of the genetically blocked reaction.

For example, ability to synthesize vitamins, amino acids and other essential substances should be lost through gene mutation if our assumptions are correct. Theoretically, any such metabolic deficiency can be "by-passed" if the substance lacking can be supplied in the medium.

In terms of specific experimental practice, we have devised a procedure in which X-rayed single-spore cultures are established on a so-called "complete" medium, i.e., one containing as many of the normally synthesized constituents of the organisms as is practicable. Subsequently these are tested

by transferring them to a "minimal" medium, i.e., one requiring the organism to carry on all the essential syntheses of which it is capable. Any loss of ability to synthesize an essential substance present in the complete medium and absent in the minimal medium is indicated by a strain growing on the first and failing to grow on the second medium. Such strains are then tested in a systematic manner to determine what substance or substances they are unable to synthesize.

Following this method, three mutant strains have been established. In one of these the ability to synthesize vitamin B_6 has been wholly or largely lost. In a second the ability to synthesize half of the vitamin B_1 molecule is absent, and in the third para-aminobenzoic acid is not synthesized. It is therefore clear that all of these substances are essential growth factors for *Neurospora*.

The preliminary results appear to us to indicate that the approach outlined may offer considerable promise as a method of learning more about how genes regulate development and function. For example, it should be possible, by finding a number of mutants unable to carry out a particular step in a given synthesis, to determine whether only one gene is ordinarily concerned with the immediate regulation of a given specific chemical reaction.

In the Proceedings of the National Academy of Sciences, Vol. 27, 1941, Pp. 499-506.

Guide to Paper by George W. Beadle and Edward L. Tatum

1. *What was Beadle and Tatum's hypothesis?*
2. *What problem did they attempt to solve?*
3. *What methods did they use to solve this problem?*
4. *What results did they obtain?*
5. *What new problem do Beadle and Tatum suggest for investigation?*

James D. Watson and Francis H. C. Crick—Discovering the Double Helix

During the early 1950s, scientists in several countries were engaged in a race to solve the mystery of heredity. In England, a 23-year-old American, James D. Watson, was working with a British colleague, Francis H.C. Crick, on this problem. Although they performed practically no experiments, they used their extraordinary ability to think and reason to win the race.

James D. Watson (1928-) was born in Chicago, and as a

young boy spent many weekends bird-watching with his father. At the age of 10 he was staying up nights reading the *World Almanac*, acquiring knowledge that he was later to use as a "Quiz Kid" on a radio show. He was a college student at the age of 15 attracted to science as a result of reading Sinclair Lewis' *Arrowsmith*. His college degree from the University of Chicago was in zoology, and he completed his PhD at Indiana University. One of his professors at Indiana University was the noted biologist Hermann Muller, who influenced Watson to engage in further study about the nature of the gene. Watson felt that the most active research on genes and heredity was taking place in England. He therefore, took a position at Cambridge University where he met Francis Crick.

Francis Crick (1916-) was educated at English universities as a physicist. During World War II, he contributed to the development of radar. His interests changed, however, after the war, and Crick then began his study of molecular biology. At Cambridge University, Crick was known as an outgoing person and one who had little patience for sloppy thinking.

The linking up of Watson with Crick created a powerful team. Their two minds fit together so that the strengths of one made up for the weaknesses of the other. After two years of model building, Watson and Crick made their breakthrough in 1953 and proposed a structure for DNA. Their discovery has had the same impact upon biology as the discovery of the structure of the atom has had on chemistry. Both Watson and Crick were awarded the Nobel Prize in 1962. Watson's book *The Double Helix* describes the process by which he and Crick won the race.

GENETICAL IMPLICATIONS OF THE STRUCTURE OF DEOXYRIBONUCLEIC ACID

JAMES D. WATSON AND FRANCIS H.C. CRICK (1953)

The importance of deoxyribonucleic acid (DNA) within living cells is undisputed. It is found in all dividing cells, largely, if not entirely, in the nucleus, where it is an essential constituent of the chromosomes. Many lines of evidence indicate that it is the carrier of a part of (if not all) the genetic specificity of the chromosomes and thus of the gene itself.

Until now, however, no evidence has been presented to show how it might carry out the essential operation required of a genetic material, that of exact self-duplication.

We have recently proposed a structure for deoxyribonucleic acid which, if correct, immediately suggests a mechanism for its self-duplication. X-ray evidence obtained by the workers at King's College, London, and presented at the same time, gives qualitative support to our structure and is incompatible with all previously proposed structures. Though the structure will not be completely proved until a more extensive comparison has been made with the X-ray data, we now feel sufficient confidence in its general correctness to discuss its genetical implications.

The chemical formula of deoxyribonucleic acid is now well established. The molecule is a very long chain, the backbone of which consists of a regular alternation of sugar and phosphate groups. To each sugar is attached a nitrogenous base which can be of four different types. Two of the possible bases—adenine and guanine—are purines, and the other two—thymine and cytosine—are pyrimidines. So far as is known, the sequence of bases along the chain is irregular. The basic unit, consisting of phosphate, sugar, and base, is known as a nucleotide. The first feature of our structure which is of biological interest is that it consists not of one chain, but of two. These two chains are both coiled around a common axis. It has often been assumed that since there was only one chain in the chemical formula there would be only one in the structural unit. However, the density taken with the X-ray evidence suggests very strongly that there are two. The other biologically important feature is the manner in which the two chains are held together. This is done by hydrogen bonds between the bases. The bases are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other. The important point is that only certain pairs of bases will fit into the structure. One member of a pair must be a purine and the other a pyrimidine, in order to bridge between the two chains. If a pair consisted of two purines, for example, there would not be room for it.

The only pairs of bases possible are:

adenine with thymine
guanine with cytosine

A given pair can be either way round. Adenine, for example, can occur on either chain; but when it does, its partner on the other chain must always be thymine.

This pairing is strongly supported by the recent analytical results which show that for all sources of deoxyribonucleic acid examined, the amount of adenine is close to the amount of thymine, and the amount of guanine is

close to the amount of cytosine. The phosphate-sugar backbone of our model is completely regular, but any sequence of the pairs of bases can fit into the structure. It follows that in a long molecule many different permutations are possible, and it therefore seems likely that the precise sequence of the bases is the code which carries the genetical information. If the actual order of the bases on one of the pair of chains were given, one could write down the exact order of the bases on the other, because of the specific pairing. Thus one chain is, as it were, the complement of the other, and it is this feature which suggests how the deoxyribonucleic acid molecule might duplicate itself.

Previous discussions of self-duplication have usually involved the concept of a template, or mold. Either the template was supposed to copy itself directly or it was to produce a negative, which in its turn was to act as a template and produce the original "positive" once again. In no case has it been explained in detail how it would do this in terms of atoms and molecules. Now our model for deoxyribonucleic acid is, in effect, a *pair* of templates, each of which is complementary to the other. We imagine that prior to duplication the hydrogen bonds are broken, and the two chains unwind and separate. Each chain then acts as a template for the formation on to itself of a new companion chain, so that eventually we shall have two pairs of chains, where we only had one before. Moreover, the sequence of the pairs of bases will have been duplicated exactly.

A study of our model suggests that this duplication could be done most simply if the single chain takes up the helical configuration. We imagine that at this stage in the life of the cell, free nucleotides are available in quantity. From time to time the base of a free nucleotide will join up by hydrogen bonds to one of the bases in the chain already formed.

Our model suggests a possible explanation for a number of other phenomena. For example, spontaneous mutation may be due to a base occasionally occurring in one of its less likely positions. Again, the pairing between homologous chromosomes at meiosis may depend on pairing between specific bases. We shall discuss these ideas in detail elsewhere.

For the moment, the general scheme we have proposed for the reproduction of deoxyribonucleic acid must be regarded as speculative. Even if it is correct, it is clear from what we have said that much remains to be discovered before the picture of genetic duplication can be described in detail. What are the nucleotide precursors? What makes the pair of chains unwind and separate? What is the precise role of the protein? Is the chromosome one long pair of deoxyribonucleic acid chains, or does it consist of patches of the acid joined together by protein?

Despite these uncertainties we feel that our proposed structure for deoxyribonucleic acid may help to solve one of the fundamental biological problems—the molecular basis of the template needed for genetic replication.

The hypothesis we are suggesting is that the template is the pattern of bases formed by one chain of the deoxyribonucleic acid and that the gene contains a complementary pair of such templates.

Nature 171:955-956; May 30 1953.

Guide to Paper by James D. Watson and Francis H.C. Crick

1. *What experimental evidence supports the structure of DNA as proposed by Watson and Crick?*
2. *Briefly describe the structure of DNA as suggested by Watson and Crick.*
3. *According to Watson and Crick, how does DNA duplicate itself?*
4. *What biological processes might be explained using the Watson and Crick structure for DNA?*
5. *What problems relating to DNA remain to be solved?*

Vernon M. Ingram—How One Amino Acid Can Produce a Fatal Disease

We have read Beadle and Tatum's paper in which they verified the "one gene–one enzyme" theory of gene action. Since enzymes are proteins, this theory was, in effect, a "one gene–one protein" hypothesis. Each gene controls the synthesis of a protein.

The work of Watson and Crick led to the idea that the substance of the gene is DNA, and therefore DNA controls the making of protein. A fellow worker of Watson and Crick at Cambridge University, Vernon M. Ingram, attempted to test this hypothesis.

After completing his PhD in 1949 at the University of London, Ingram developed a method for analyzing the hemoglobin molecule. His attention was drawn to sickle-cell anemia, a hereditary blood disease caused by the mutation of a single gene. The red blood cells of people with this disease contain an abnormal type of hemoglobin. When these blood cells give up their oxygen to the cells of the body, they become sickle- or crescent-shaped. These sickle cells are very weak and are easily destroyed; thus the number of circulating red blood cells is reduced along with the supply of oxygen to the cells. This disease is found almost entirely among Black members of the population.

Ingram attempted to find how the mutation of a single gene could cause a precise change in the protein hemoglobin. His discovery, reported in the following paper, supports the idea that genes contain the information to synthesize proteins.

A SPECIFIC CHEMICAL DIFFERENCE BETWEEN THE GLOBINS OF NORMAL HUMAN AND SICKLE-CELL ANEMIA HEMOGLOBIN

VERNON M. INGRAM (1956)

Of all the abnormal human hemoglobins, the one that has been most intensively studied is hemoglobin *S* from patients with sickle-cell anemia.

Hemoglobin is still too large a molecule for detailed analysis of amino-acid sequence. However, it was thought that if a rapid method could be found of characterizing the chemical properties of the peptides in a tryptic digest, then perhaps a replacement of even a single residue for another might be detected without elaborate analysis.

The action of trypsin on proteins is at present the most reliable way of splitting a peptide chain at specific peptide bonds. The enzyme attacks only those bonds which are derived from the carboxyl group of the amino-acids lysine and arginine. There are about sixty of these in the hemoglobin *A* and *S* molecule, but since it is expected that each molecule is composed of two identical half-molecules, the number of peptides obtained by the action of trypsin should be about thirty, with an average chain-length of ten amino-acids. Small differences in the two proteins will result in small changes in one or more of these peptides. These should be detectable when the mixture is examined by a two-dimensional combination of paper electrophoresis and paper chromatography. It was decided to call the resulting chromatogram the "fingerprint" of the protein.

To prepare such a "fingerprint," samples of purified hemoglobins *A* and *S* were denatured by heat at 90°C for 4 minutes and digested with trypsin (2 percent by weight) at pH 8 and 37°C for 43 hours and subjected to electrophoresis. Ascending chromatography with *n*-butyl alcohol/acetic acid/water produced chromatograms. Reproducibility was often good enough to superimpose the spots belonging to similar peptides in the two chromatograms.

The "fingerprints" show approximately thirty peptide spots, as was expected from the amino-acid composition. This confirms the view that the human hemoglobin molecule consists of two identical half-molecules. The same number of peptides was found for both hemoglobins, as was to be expected from their containing the same number of lysine and arginine

residues. Most of the peptides are well resolved, and appear to be similar in the two proteins. However, there is one peptide spot clearly visible in the digest of hemoglobin *S* which is not obvious in the hemoglobin *A* "fingerprint." Apart from its position, this peptide is characterized by its orange-colour reaction.

Summarizing the results of the electrophoretic and chromatographic examination, it can be seen that there is one peptide among the thirty or so which in hemoglobin *S* is positively charged, but which is uncharged in digests of hemoglobin *A*. There is also a small change in the chromatographic behaviour of the peptide.

One can now answer at least partly the question put earlier, and say that there is a difference in the amino-acid sequence in one small part of one of the polypeptide chains. This is particularly interesting in view of the genetic evidence that the formation of hemoglobin *S* is due to a mutation in a single gene. It remains to be seen exactly how large a portion of the chains is affected and how the sequences differ.

Nature 178: 792-794; October 13, 1956.

GENE MUTATIONS IN HUMAN HEMOGLOBIN: THE CHEMICAL DIFFERENCE BETWEEN NORMAL AND SICKLE-CELL HEMOGLOBIN

VERNON M. INGRAM (1957)

I reported recently that normal and sickle-cell anemia human hemoglobins differ only in a small portion of their polypeptide chains. The latter is an abnormal protein which is inherited in a strictly Mendelian manner; it is now possible to show, for the first time, the effect of a single gene mutation as a change in one amino-acid of the hemoglobin polypeptide chain for the manufacture of which that gene is responsible.

In previous experiments, tryptic digests of the two proteins had been prepared; the resulting mixtures of small peptides were separated on a sheet of paper, using electrophoresis in one direction and partition chromatography in the other. These paper chromatograms derived from the two proteins, which I had called "fingerprints," showed all peptides to have identical electrophoretic and chromatographic properties, except for one spot, peptide No. 4. This occupied different positions in the "fingerprints" of normal (*A*) and sickle-cell anemia (*S*) hemoglobins, indicating that the difference between the two proteins was located there. I have now determined the chemical constitution of these No. 4 peptides derived from both hemoglobin *A* and *S*.

In both cases qualitative amino-acid analysis by paper chromatography

showed the presence of histidine, valine, leucine, threonine, proline, glutamic acid and lysine. There was more glutamic acid in the hemoglobin *A* peptide, but more valine in the hemoglobin *S* peptide.

The only difference found between the two No. 4 peptides is that the first glutamic acid residue of the hemoglobin *A* peptide is replaced by valine in the hemoglobin *S* peptide.

It is known from X-ray crystallographic and from chemical studies that the human hemoglobin molecule of molecular weight 66,700 is composed of two identical half-molecules, each approximately 33,000. It is believed that this substitution, which occurs in each of the two identical half-molecules, constitutes the only chemical difference between normal and sickle-cell anemia hemoglobins. Certainly the heme groups of the two proteins are the same. The fact that in each half-molecule a glutamic acid is replaced by the neutral amino-acid valine agrees with previous findings. All the other peptides of the tryptic digest occupy identical, and characteristic, positions in the two "fingerprints." Qualitative amino-acid analyses of these peptides have now been carried out, but have failed to reveal any differences between them. It would seem probable, therefore, that they have identical structures, leaving the two No. 4 peptides as the only ones that differ.

We owe to Pauling and his collaborators the realization that sickle-cell anemia is an example of an inherited "molecular disease" and that it is due to an alteration in the structure of a large protein molecule, an alteration leading to a protein which is by all criteria still a hemoglobin. It is now clear that, per half-molecule of hemoglobin, this change consists in replacement of only one of nearly 300 amino-acids, namely, glutamic acid, by another, valine—a very small change indeed.

Nature 180:326-328; August 17, 1957.

Guide to Papers by Vernon M. Ingram

1. *What problems did Ingram attempt to solve?*
2. *What steps were necessary for Ingram to follow in order to solve these problems?*
3. *Why did Ingram use trypsin in his experiment?*
4. *What differences between normal hemoglobin and sickle hemoglobin did Ingram find?*
   a. *In his 1956 paper?*
   b. *In his 1957 paper?*
5. *What conclusions did Ingram draw from his experiments?*
6. *What questions raised by Ingram's work remain to be answered?*

Arthur Kornberg—Making DNA in a Test Tube

Arthur Kornberg (1918-) was known as the smartest kid on the block when he was a boy in Brooklyn. He was graduated from high school when he was 16, and he received his bachelor's degree when only 19. After completing studies in medicine, Kornberg decided to devote his life to biochemical research instead of medical practice.

He worked in several laboratories, where his discoveries in enzyme chemistry won him many awards. Although Kornberg is soft spoken and unassuming, his intelligence and sense of organization help him to mobilize all of his energies to attack a problem.

We have read about the model of DNA proposed by Watson and Crick. After scientists read about this model, they attempted to make DNA duplicate itself outside the living cell. How Kornberg succeeded in doing this is described in the following paper.

For his work with DNA, Kornberg received the Nobel Prize in 1959 and is still actively involved in this field.

His wife is also a biochemist, and they have collaborated on many scientific papers. Despite his intense interest in research, Kornberg attends all the home football games at Stanford University and is a baseball fan of the San Francisco Giants.

BIOLOGIC SYNTHESIS OF DEOXYRIBONUCLEIC ACID
AN ISOLATED ENZYME CATALYZES SYNTHESIS OF THIS NUCLEIC ACID IN RESPONSE TO DIRECTIONS FROM PRE-EXISTING DNA

ARTHUR KORNBERG (1960)

Knowledge drawn in recent years has just about convinced most of us that deoxyribonucleic acid (DNA) is the genetic substance. We shall assume then that it is DNA which not only directs the synthesis of the proteins and the development of the cell but which must also be the substance which is copied so as to provide for a similar development of the progeny of that cell for many generations. Deoxyribonucleic acid, like a tape recording, carries a message in which there are specific instructions for a job to be done. Also,

exact copies can be made from it, as from a tape recording, so that this information can be used again and elsewhere in time and space.

Although we have in the Watson and Crick proposal a mechanical model of replication, we may at this point pose the question: What is the chemical mechanism by which this super molecule is built up in the cell?

The following are the requirements for the synthesis of DNA using a DNA-synthesizing enzyme, purified from the bacteria *Escherichia coli*.

All four of the nucleotides which form the adenine-thymine and guanine-cytosine couples must be present. The substrates must be the tri- and not the diphosphates, and only the deoxy sugar compounds are active. Deoxyribonucleic acid, which must be present, may be obtained from animal, plant, bacterial, or viral sources, and the best indications are that all these DNA samples serve equally well in DNA synthesis provided their molecular weight is high. The product accumulates until one of the substrates is exhausted and may be 20 or more times greater in amount than the DNA added, and thus is composed to the extent of 95 percent or more of the substrates added to the reaction mixture.

The unifying and basic generalization about the action of the bacterial enzyme is that it catalyzes the synthesis of a new DNA chain in response to directions from a DNA template. These directions are dictated by the hydrogen bonding relationship of adenine to thymine and of guanine to cytosine. The experimental bases for this conclusion are derived from the observations of: the double-stranded molecule and the requirement for all four bases (adenine, thymine, guanine, cytosine) and DNA to be present.

Science 131:1503-1508; May 20, 1960.

Guide to Paper by Arthur Kornberg

1. *What problem did Kornberg attempt to solve?*
2. *What method did Kornberg use to solve this problem?*
3. *What results did Kornberg obtain?*
4. *Why did Kornberg use the bacterial enzyme?*
5. *What problems can be attacked now that Kornberg's work is complete?*

THE TRANSFER OF TRAITS–SUMMARY QUESTIONS

1. *What was the contribution of each investigator in this section to our understanding of how traits are passed on from parent to offspring?*
2. *Project yourself 50 years into the future.*
   a. *How might we be using our knowledge of genetics then?*
   b. *How do you feel about such uses of genetics?*
3. *Read the book Brave New World by Aldous Huxley. Discuss what you have read in this section in relation to the situations in the novel. .*

OBSERVATION AND EXPERIMENTATION

Claude Bernard—How Experimental Ideas are Produced from Casual Observation

After reading Bernard's paper, prepare a series of questions which you feel will help a friend understand the important points in the paper.

WHERE THE STARTING POINT FOR EXPERIMENTAL RESEARCH IS AN OBSERVATION

CLAUDE BERNARD (1865)

Experimental ideas are often born by chance, with the help of some casual observation. Nothing is more common; and this is really the simplest way of beginning a piece of scientific work. We can take a walk, so as to speak, in the realm of science, and we pursue what happens to present itself to our eyes. Bacon compares scientific investigation with hunting; the observations that present themselves are the game. Keeping the same simile, we may add that, if the game presents itself when we are looking for it, it may also present itself when we are not looking for it, or when we are looking for game of another kind.

One day, rabbits from the market were brought into my laboratory. They were put on the table where they urinated, and I happened to observe that their urine was clear and acid. This fact struck me, because rabbits, which are herbivora, generally have turbid and alkaline urine; while on the other hand carnivora, as we know, have clear and acid urine. This observation of acidity in the rabbits gave me an idea that these animals must be in the nutritional condition of carnivora. I assumed that they had probably not eaten for a long time, and that they had been transformed by fasting, into veritable carnivorous animals, living on their own blood. Nothing was easier than to verify this preconceived idea or hypothesis by experiment.

I gave the rabbits grass to eat; and a few hours later, their urine became turbid or alkaline. I then subjected them to fasting and after 24 hours or 36

hours at most, their urine again became clear and strongly acid; then after eating grass, their urine became alkaline again, etc. I repeated this very simple experiment a great many times, and always with the same result. I then repeated it on a horse, an herbivorous animal which also has turbid and alkaline urine. I found that fasting, as in rabbits, produces prompt acidity of the urine, with such an increase in urea, that it spontaneously crystallizes at times in the cooled urine. As a result of my experiments, I thus reached the general proposition which then was still unknown, to wit, that all fasting animals feed on meat, so that herbivora then have urine like that of carnivora.

We are here dealing with a very simple, particular fact which allows us easily to follow the evolution of experimental reasoning. When we see a phenomenon which we are not in the habit of seeing, we must always ask ourselves what it is connected with, or putting it differently, what is its proximate cause; the answer or the idea, which presents itself to the mind, must then be submitted to experiment. When I saw the rabbit's acid urine, I instinctively asked myself what could be its cause.

The experimental idea consisted in the connection, which my mind spontaneously made, between acidity of the rabbit's urine, and the state of fasting which I considered equivalent to a true flesh-eater's diet. The inductive reasoning which I implicitly went through was the following syllogism: the urine of carnivora is acid; now the rabbits before me have acid urine; therefore they are carnivora, i.e., fasting. This remained to be established by experiment.

But to prove that my fasting rabbits were really carnivorous, a counterproof was required. A carnivorous rabbit had to be experimentally produced by feeding it with meat, so as to see if its urine would then be clear, as it was during fasting. So I had rabbits fed on cold boiled beef (which they eat very nicely when they are given nothing else). My expectation was again verified, and, as long as the animal diet was continued, the rabbits kept their clear and acid urine.

To complete my experiment, I made an autopsy on my animals, to see if meat was digested in the same way in rabbits as in carnivora. I found, in fact, all the phenomena of an excellent digestion in their intestinal reactions.

An Introduction to the Study of Experimental Medicine, Henry Schuman, New York, 1949.